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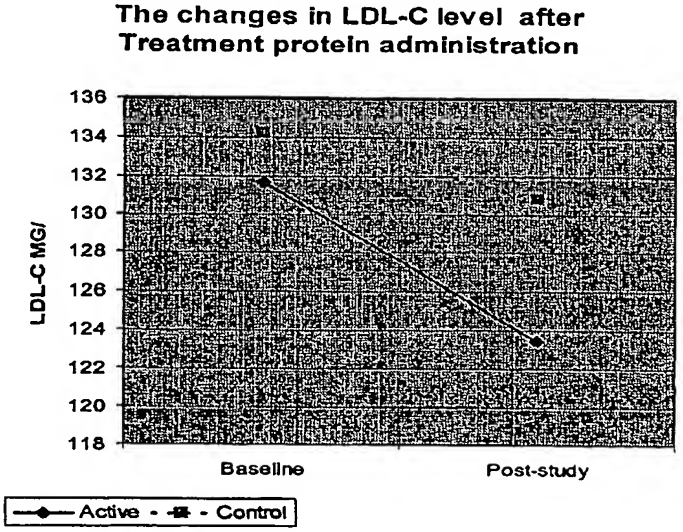
(54) Title: REDUCING CHOLESTEROL WITH HYDROLYZED WHEY PROTEIN



(57) Abstract: The present invention provides a use of a whey protein hydrolysate in the manufacture of a medicament or an edible product useful to lower low-density ipoprotein (LDL) cholesterol in a mammal. The present invention also provides an article, including: a) an edible product including a whey protein hydrolysate; and b) packaging material, wherein the packaging material is marked to indicate that the product gives the benefit of lowering cholesterol. The present invention further provides methods and treatments for a mammal to lower low-density lipoprotein (LDL) cholesterol, including feeding to the mammal a whey protein hydrolysate in amounts and at intervals effective to lower LDL cholesterol.

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REDUCING CHOLESTEROL WITH HYDROLYZED WHEY PROTEIN

Cross-Reference to Related Applications

This patent application claims the benefit of priority, under 35 U.S.C. Section 119(e), to U.S. Provisional Patent Application Serial Number 60/352,014, filed on January 25, 2002, and to U.S. Provisional Patent Application Serial Number 60/366,902, filed on March 22, 2002, the specifications of which are herein incorporated by reference.

Field of the Invention

The present invention relates to methods and products useful for lowering cholesterol in mammals.

Background of the Invention

Cholesterol has also been associated with a variety of diseases, including cardiovascular diseases. (American Heart Association, *2001 Heart and Stroke Statistical Update*. Dallas, Tex.: American Heart Association, 2000) A high level of low-density lipid (LDL) cholesterol has been identified as a risk factor for coronary diseases. In contrast, a lower level of LDL cholesterol has a protective effect on cardiovascular disease.

Hypertension is the most prevalent risk factor for cardiovascular disease in this country, affecting over 50 million Americans. (American Heart Association. *2001 Heart and Stroke Statistical Update*. Dallas, Tex.: American Heart Association, 2000) Antihypertensive medications can be helpful in lowering blood pressure, however many can cause significant side effects. One family of medications that appears to induce fewer side effects includes the angiotensin-converting-enzyme (ACE) inhibitors. (American Heart Association. *Hypertension Primer; The Essentials of High Blood Pressure; Basic Science, Popular Science, and Clinical Management*. Ed. Izzo, JL and Black, HR. 2nd edition, Dallas, Tex.: 1999) However, these medications are not risk free and are costly. (Moser, M., *Am J Hypertens*. 1998;11:120S-127S) Recently, numerous *in vitro* and a few animal trials have indicated that some food-derived peptides might also inhibit ACE and subsequently result in

significant reductions in blood pressure. (WO 01/85984; Fujita et al., *J Food Sci.* 2000;65:564-569; Santure M, Pitre M, and Bachelard H. Antihypertensive effects of whey protein hydrolysates in conscious, unrestrained spontaneously hypertensive rats. First and second studies, unpublished data.) Some hydrolyzed whey proteins were shown to have significant related activity while unmodified whey proteins were not. (WO 01/85984) Moreover, recent drug trials indicate that ACE inhibition might result in other significant health benefits such as improved cardiac function, insulin sensitivity and possible prevention of dementia. (*N Engl J Med.* 2000;342:145- 153; Mancini GB., *Clin Invest Med.* 2000;23:144-161; Breo et al., *Arch Neurol.* 2000;57:1586-1591)

Therefore, in order to treat or prevent a variety of diseases, methods and products are needed to reduce LDL cholesterol or to maintain lower, recommended levels of LDL cholesterol. In some circumstances, it would also be beneficial for these methods and products to reduce blood pressure.

Summary of the Invention

The present invention provides the use of a whey protein hydrolysate in the manufacture of a medicament useful to lower low-density lipoprotein (LDL) cholesterol in a mammal.

The present invention also provides the use of a whey protein hydrolysate in the manufacture of an edible product useful to lower low-density lipoprotein (LDL) cholesterol in a mammal.

The present invention also provides an article, comprising: a) an edible product comprising a whey protein hydrolysate; and b) packaging material, wherein the packaging material is marked to indicate that the product gives the benefit of lowering cholesterol.

The present invention further provides a treatment for a mammal to lower low-density lipoprotein (LDL) cholesterol, comprising feeding to the mammal a whey protein hydrolysate in amounts and at intervals effective to lower LDL cholesterol.

The present invention also provides a method to lower low-density lipoprotein (LDL) cholesterol in a mammal in need of such treatment, comprising feeding to the mammal a whey protein hydrolysate in amounts and at

intervals effective to lower LDL cholesterol.

Brief Description of the Figures

Figure 1 depicts systolic blood pressure (SBP) readings taking during the dosing evaluation using a dose of 20 grams of protein, as described in Example 1. "Active" (or A) refers to the Test Treatment Protein, BioZate 1® hydrolyzed whey protein isolate, and "Control" (or C) refers to the Comparison Treatment Protein, *Bi*PRO® whey protein isolate.

Figure 2 depicts diastolic blood pressure (DBP) readings taking during the dosing evaluation using a dose of 20 grams of protein, as described in Example 1. "Active" (or A) refers to the Test Treatment Protein, BioZate 1® hydrolyzed whey protein isolate, and "Control" (or C) refers to the Comparison Treatment Protein, *Bi*PRO® whey protein isolate.

Figure 3 depicts systolic blood pressure (SBP) readings taking during the dosing evaluation using a dose of 10 grams of protein, as described in Example 1. "Active" (or A) refers to the Test Treatment Protein, BioZate 1® hydrolyzed whey protein isolate, and "Control" (or C) refers to the Comparison Treatment Protein, *Bi*PRO® whey protein isolate.

Figure 4 depicts diastolic blood pressure (DBP) readings taking during the dosing evaluation using a dose of 10 grams of protein, as described in Example 1. "Active" (or A) refers to the Test Treatment Protein, BioZate 1® hydrolyzed whey protein isolate, and "Control" (or C) refers to the Comparison Treatment Protein, *Bi*PRO® whey protein isolate.

Figure 5 depicts systolic blood pressure (SBP) readings taking during the dosing evaluation using a dose of 5 grams of protein, as described in Example 1. "Active" (or A) refers to the Test Treatment Protein, BioZate 1® hydrolyzed whey protein isolate, and "Control" (or C) refers to the Comparison Treatment Protein, *Bi*PRO® whey protein isolate.

Figure 6 depicts diastolic blood pressure (DBP) readings taking during the dosing evaluation using a dose of 5 grams of protein, as described in Example 1. "Active" (or A) refers to the Test Treatment Protein, BioZate 1® hydrolyzed whey protein isolate, and "Control" (or C) refers to the Comparison Treatment Protein, *Bi*PRO® whey protein isolate.

Figure 7 depicts systolic blood pressure (SBP) readings taken during the pilot trial, as described in Example 1.

Figure 8 depicts diastolic blood pressure (DBP) readings taken during the pilot trial, as described in Example 1.

Figure 9 depicts the changes in total cholesterol level during the pilot trial, as described in Example 1.

Figure 10 depicts the changes in LDL cholesterol level during the pilot trial, as described in Example 1.

Figure 11 depicts the changes in HDL cholesterol level during the pilot trial, as described in Example 1.

Figure 12 depicts the changes in triglyceride (TG) during the pilot trial, as described in Example 1.

Figure 13 depicts the side effects during the pilot trial, as described in Example 1.

Figure 14 depicts the hypertension and ACE inhibition side effects during the pilot trial, as described in Example 1.

Figure 15 depicts the gastrointestinal side effects during the pilot trial, as described in Example 1.

Figure 16 depicts alkaline phosphatase (AP) level during the pilot trial, as described in Example 1.

Figure 17 depicts aspartate amino transferase (AST) level during the pilot trial, as described in Example 1.

Figure 18 depicts amino alanine transferase (ALT) level during the pilot trial, as described in Example 1.

Figure 19 depicts the reverse phase high performance liquid chromatography (RP-HPLC) profile of UF-BioZate, as described in Example 3.

Figure 20 depicts the RP-HPLC profiles of fractions F3 (Figure 20A) and F5 (Figure 20B) of UF-BioZate, as described in Example 3.

Detailed Description of the Invention

Subjects were treated with BioZate 1® hydrolyzed whey protein isolate or *Bi*PRO® whey protein isolate. Treatment with the hydrolyzed whey protein isolate was found to reduce LDL cholesterol and to reduce blood pressure.

Whey protein

The hydrolyzed whey protein useful in the practice of the invention can be produced from a solution of whey protein, for example, from a solution of a sample of whey protein. In some embodiments of the invention, the whey protein sample can be a whey protein fraction, a whey protein concentrate, or a whey protein isolate. A "whey protein concentrate" is characterized by having a protein content of at least about 25% protein. A "whey protein isolate" is characterized by having a protein content of at least about 90% protein. While a whey protein isolate having the characteristics of *Bi*PRO® whey protein isolate is one source of whey protein, other sources of whey protein can also be used in the practice of the invention. Whey protein can be obtained from commercial-scale fractionation of cheese whey by various processes, including ion-exchange processing using cationic and/or anionic resins selected for the intended functionality of the isolate. (Pearce, R. J., 1992, Whey protein recovery and whey protein fractionation, Whey and Lactose Processing, JG Zadow, Ed., Elsevier, London, 271-316.) Commercial whey protein isolate products issued from ion-exchange processing, such as *Bi*PRO® whey protein isolate are typically characterized by a high protein content (≥94% w/w), low ash content (<3%), and traces (<1%) of fat and lactose.

As noted, other sources of whey protein than *Bi*PRO® whey protein isolate can be used. The whey protein may have similar analyses to that of *Bi*PRO® whey protein isolate, for example, by varying by from 0 to 25%, e. g., from 5 to 10%, or less, from the Typical Range values for *Bi*PRO® whey protein isolate. A suitable whey protein can be produced having similar properties through a selective ion exchange process. Such a process is described in U. S. Patent No. 4,154,675 to Jowett, et al., and U. S. Patent No. 4,218,490 to Phillips, et al. If properly produced, whey protein samples having lower protein contents, e. g., as low as 35%, can be used.

*Bi*PRO® whey protein isolate is available from Davisco Foods International, Inc., with offices at 11000 W. 78th Street, Suite 210, Eden Prairie, Minnesota 55344. The preferred *Bi*PRO® whey protein isolate has a Protein Digestibility Corrected Amino Acid Score (PDCAAS) of 1.14. The fat and lactose levels are less than 1%. The *Bi*PRO® whey protein isolate is prepared

by ion-exchange technology, and contains about 55-65% (w/w) β-lactoglobulin. Preferably, the whey protein isolate employed according to the invention will contain at least 55% and preferably at least 60%, β-lactoglobulin, with the remaining including α-lactalbumin, serum albumin, and immunoglobulins.

*Bi*PRO® whey protein isolate is essentially undenatured, is fully soluble in water over the pH range 2.0 to 9.0 and has the following analysis:

Analysis*	**Specification**	**Typical Range**	**Test Method**
Moisture (%)	5.0 max.	4.9 ± 0.1	Vacuum Oven
Protein, dry basis (N x 6.38) (%)	95.0 min.	97.8 ± 0.4	Leco Combustion
Fat (%)	1.0 max.	0.3 ± 0.1	Mojonnier
Ash (%)	3.0 max.	2.0 ± 0.3	Gravimetric
Lactose (%)	1.0 max.	<0.5	By Difference
pH	6.7 - 7.5	7.2 ± 0.1	10% Sol. @ 20°C

*All results are reported "AS IS" basis except where noted. Methods are based on "Standard Methods for the Examination of Dairy Products, 16th Edition.

On a more detailed analysis of *Bi*PRO® whey protein isolate, the following is found for each 100 grams of whey protein isolate:

Component	
Protein (g)	93
Total Fat (g)	0.3
Saturated Fat (g)	0.2
Cholesterol (mg)	10
Total Carbohydrates (g)	0
Dietary Fiber (g)	0
Sugars (g)	0
Moisture (g)	5
Ash (g)	2
Sodium (mg)	600
Potassium (mg)	120

To provide an amino acid profile of the *Bi*PRO® whey protein isolate, samples were subjected to drying for 24 hours in a desiccator over phosphorous pentoxide and sodium hydroxide. The dry samples were subjected to vapor phase hydrolysis by 6N HCl at 110° Celsius for 24 hours under argon atmosphere in the presence of phenol. The samples were subsequently reconstituted in sodium borate buffer and analyzed with post-column reaction with ninhydrin. The amino acids were separated and analyzed on a Beckman 6300 Amino Acid Analyzer. Norleucine was used as an internal standard. Tryptophan decomposes under these acid hydrolysis conditions. Therefore, tryptophan was determined after alkaline hydrolysis in sealed tubes and subsequent amino acid analysis using a reduced program for basic amino acids only. The analysis showed the following:

Amino Acid	Grams per 100 g powder
Alanine	4.75
Arginine	1.60
Aspartic Acid	10.91
Cystine	3.02
Glutamic Acid	15.38
Glycine	1.65
Histidine	2.07
Isoleucine	4.59
Leucine	12.24
Lysine	10.50
Methionine	2.04
Phenylalanine	3.44
Proline	3.89
Serine	3.44
Threonine	4.54
Tryptophan	2.62
Tyrosine	1.72
Valine	4.59

Hydrolysis of whey protein

The whey protein is hydrolyzed to prepare the whey protein hydrolysate. In some embodiments of the invention, the enzyme trypsin is used, either alone or in combination with other enzymes, to hydrolyze the whey protein. Other enzymes that may be used in the hydrolysis include, but are not limited to those disclosed in "Biochemical Pathways", edited by Gerhard Michal (1999), the disclosure of which is herein incorporated by reference. For example, additional enzymes can include endopeptidases or exopeptidases, and the serine, cysteine, aspartate and metallo peptidases.

To prepare the hydrolyzed whey protein used in the Examples, *Bi*PRO® whey protein isolate was solubilized at 20% w/v, adjusted to pH 8.0 or 8.5 by

using a mixture of NaOH and KOH 4N and maintained at temperatures between 40°C and 50°C corresponding to the optimal temperature of the trypsin enzyme. The characteristics of the enzyme used for the preparation of the enzymatic hydrolysate for the study are shown below:

Characteristics of the enzyme source used for the preparation of BioZate hydrolysate

Enzyme (Name, Supplier)	Source	Optimal pH	Temp. (°C)	Hydrolysate
Trypsin VI Trypsin Activity 2,400 U/mg minimum *Canadian Inovatech Inc Abbotsford, BC, Canada*	Porcine	8.0	37	BioZate 1® hydrolyzed whey protein isolate

The enzymatic hydrolysis was performed under pH-stat conditions until a degree of hydrolysis (DR) of 5.5-6.5% for BioZate 1® hydrolyzed whey protein isolate was reached. The hydrolysis reaction was stopped at the selected DH values by means of heat treatment (75 to 85°C for 15 s) in a plate heat exchanger to inactivate the enzyme and followed by cooling and storage at 5-10°C until further processing. The resulting hydrolysate was further spray dried and handled as powdered ingredient.

Whey protein hydrolysates

Hydrolysis of whey protein produces hydrolyzed whey protein. The hydrolysate may optionally be fractionated and/or dried. The hydrolysate can be used in the manufacture of a medicament or an edible product. The hydrolysate may also be fed to mammals, for example, in an isolated form or as an ingredient in a medicament or edible product. The typical characteristics of the hydrolysate used are shown below.

BioZate 1® hydrolyzed whey protein isolate

Analysis	Specification	Typical Range	Test Method
Moisture (%)	5.5 max.	5.2 ± 0.3	Vacuum Oven
Protein, dry basis (N x 6.38) (%)	90.0 min.	91.0 ± 0.5	Leco Combustion
Amino Nitrogen (AN) (%)	1.5 min.	1.8 ± 0.3	Formol Titration
AN/TN (%)	11.8 min.	12.8 ± 1.0	Calculated
Degree of Hydrolysis (%)	4.0 min.	5.5 ± 1.5	OPA Method
Fat (%)	1.0 max.	<1.0	Mojonnier
Ash (%)	6.0 max.	5.5 ± 0.3	Gravimetric
Lactose (%)	1.0 max.	<1.0	By Difference
pH	8.5 max.	8.0 ± 0.2	10% Sol. @ 20°C

Molecular Weight Profile (Daltons)	Peptides
Greater than 10,000	30 – 45%
5,000 – 10,000	7 – 12%
2,000 – 5,000	15 – 25%
Less than 2,000	30 – 45%

* All results reported "AS IS" basis except where noted. Methods are from "Standard Methods for the Examination of Dairy Products, 16th Edition.

The present invention provides the use of a whey protein hydrolysate in the manufacture of a medicament useful to lower low-density lipoprotein (LDL) cholesterol in a mammal. In some embodiments, the medicament is also useful to treat hypertension in a mammal.

The present invention also provides the use of a whey protein hydrolysate in the manufacture of an edible product useful to lower low-density lipoprotein (LDL) cholesterol in a mammal. In some embodiments, the product is also

useful to treat hypertension in a mammal. In some embodiments, the edible product is a food product. In some embodiments, the food product is a beverage product, a dessert product, a confectionary product, a baked product, a dairy product, a cereal, a bread, a muffin, a cake, a health food bar or a snack bar.

The present invention also provides an article, comprising: a) an edible product comprising a whey protein hydrolysate; and b) packaging material, wherein the packaging material is marked to indicate that the product gives the benefit of lowering cholesterol. In some embodiments, the packaging material is marked to indicate that the product lowers low-density lipoprotein (LDL) cholesterol. In some embodiments, the packaging material is marked to indicate that the whey protein hydrolysate gives the benefit of lowering cholesterol. In some embodiments, the packaging material is marked to indicate that the product also gives the benefit of treating hypertension. In some embodiments, the edible product is a food product. In some embodiments, the food product is beverage product, a dessert product, a confectionary product, a baked product, a dairy product, a cereal, a bread, a muffin, a cake, a health food bar or a snack bar.

The present invention also provides a treatment for a mammal to lower low-density lipoprotein (LDL) cholesterol, comprising feeding to the mammal a whey protein hydrolysate in amounts and at intervals effective to lower LDL cholesterol. In some embodiments, the feeding of the hydrolysate also treats hypertension.

The present invention also provides a method to lower low-density lipoprotein (LDL) cholesterol in a mammal in need of such treatment, comprising feeding to the mammal a whey protein hydrolysate in amounts and at intervals effective to lower LDL cholesterol. In some embodiments, the feeding of the hydrolysate also treats hypertension.

For any of the above uses, articles, or treatments involving the hydrolyzed whey protein, the whey protein hydrolysate may be prepared according to the process of preparing a solution comprising a whey protein sample and at least one proteolytic enzyme, and holding the solution under conditions effective to partially hydrolyze the whey protein to provide a hydrolysate having LDL cholesterol lowering properties. In some embodiments, the whey protein sample is a whey protein fraction. In some embodiments, the

whey protein sample is a whey protein concentrate. In some embodiments, the whey protein sample is a whey protein isolate. In some embodiments, the whey protein sample is prepared by ion-exchange processing. In some embodiments, the whey protein sample has a Protein Digestibility Corrected Amino Acid Score of about 1.14. In some embodiments, the whey protein sample has an ash content of less than about 3%. In some embodiments, the whey protein sample has a mineral content of calcium in the range of from about 15-20 meq/kg. In some embodiments, the whey protein sample has a mineral content of magnesium of about 1 meq/kg. In some embodiments, the whey protein sample has a fat and lactose content of less than about 1%. In some embodiments, the whey protein sample contains at least about 55% β-lactoglobulin. In some embodiments, the whey protein sample has a protein content of at least 35%. In some embodiments, the whey protein sample has a protein content that is within the range of 0 to 25% of 97.8%. In some embodiments, the whey protein sample has a protein content that is within the range of 5-10% of 97.8%. In some embodiments, the whey protein sample has a protein content that varies less than 5% from 97.8%. In some embodiments, the whey protein sample has a protein content that is 97.8 ± 0.4%. In some embodiments, the whey protein sample is characterized as follows:

Analysis	Range
Moisture (%)	4.9 ± 0.1
Protein, dry basis (%)	97.8 ± 0.4
Fat (%)	0.3 ± 0.1
Ash (%)	2.0 ± 0.3
Lactose (%)	<0.5
pH	7.2 ± 0.1

. In some embodiments, the solution comprises the proteolytic enzyme trypsin. In some embodiments, the solution is hydrolyzed at a pH within the range of from 8.0-8.5. In some embodiments, the whey protein sample is solubilized at about 20% w/v. In some embodiments, the solution is held at a temperature

within the range of from 40°-50°C. In some embodiments, the hydrolysis of the whey protein sample is stopped by heat treatment. In some embodiments, the hydrolysis of the whey protein sample is stopped by ultrafiltration. In some embodiments, the hydrolysate is dried following hydrolysis. In some embodiments, the hydrolysate is spray dried following hydrolysis. In some embodiments, the hydrolysate is freeze dried following hydrolysis. In some embodiments, the whey protein hydrolysate is characterized by a degree of hydrolysis within the range of from about 5.5-6.5%. In some embodiments, the whey protein hydrolysate is characterized by the molecular weight profile:

Molecular Weight Profile (Daltons)	Peptides
Greater than 10,000	30 – 45%
5,000 – 10,000	7 – 12%
2,000 – 5,000	15 – 25%
Less than 2,000	30 – 45%

. In some embodiments, the whey protein hydrolysate is characterized as follows:

Analysis	Range
Moisture (%)	5.2 ± 0.3
Protein, dry basis (%)	91.0 ± 0.5
Amino Nitrogen (AN) (%)	1.8 ± 0.3
AN/TN (%)	12.8 ± 1.0
Degree of Hydrolysis (%)	5.5 ± 1.5
Fat (%)	<1.0
Ash (%)	5.5 ± 0.3
Lactose (%)	<1.0
pH	8.0 ± 0.2

The hydrolysate can be administered in various forms. For example, the hydrolysate may be a component of an edible product, for example, a food product. An "edible product" is a product that is consumable, but one which

need not be a part of a normal diet. A "food product" is a product that is consumable that is part of a normal diet. In some embodiments, the food product is a beverage product, a dessert product, a confectionary product, a baked product, a dairy product, a cereal, a bread, a muffin, a cake, a health food bar or a snack bar. The hydrolysate may also be administered in the form of a tablet or a capsule.

The hydrolysate is administered in amounts and at intervals effective to decrease LDL cholesterol or to maintain a lower, recommended level of LDL cholesterol. A "recommended" level of LDL cholesterol is that level can be determined by a physician depending on the specific characteristics of the patient. In general, a LDL cholesterol level less than 100 milligrams per deciliter of blood is optimal; 100-129 milligrams per deciliter of blood is above optimal, 130-159 milligrams per deciliter of blood is borderline high, 160-189 milligrams per deciliter of blood is high, and 190 milligrams per deciliter of blood and above is very high.

In some embodiments of the invention, the hydrolysate can be added to an edible product at a dosage of about 5, 10, or 20 grams per serving. Different dosages will be effective, for example, a dose of from 1-5, 5-10, 10-15, 15-20, or 20-25 grams per serving may be used. The product can be consumed at various effective intervals, for example, once, twice, or three times daily, or, for example, 1, 2, 3, 4, 5, 6, 7, 14, or 21 times per week. Further, by increasing or decreasing the intervals when the hydrolysate is consumed, it will also be able to increase or decrease the effective dosage.

The edible products can be packaged with packaging material. The packaging material can be marked to indicate that the product lowers cholesterol, for example LDL cholesterol. The packaging material can be marked to indicate that the whey protein hydrolysate gives the benefit of lowering cholesterol. The markings may be of any form to convey the information, for example, the markings may be words. In some embodiments, the markings may be symbols other than words. The packaging material may also be marked to indicate that the product, or hydrolysate, gives the benefit of treating hypertension or lowering blood pressure.

The packaging material can include instructions, for example,

instructions for the consumer to consume, eat, or drink the product in a manner so as to lower LDL cholesterol. The packaging material may include instructions to indicate that the consumption of the edible product will promote health by being a part of a regimen to lower LDL cholesterol to prevent or treat high cholesterol. The instructions may indicate the amounts and, optionally, intervals the consumer should consume the product in order to lower LDL cholesterol.

Cholesterol, for example, LDL, HDL, and total cholesterol, can be measured by methods well known to the art worker. For example, a blood sample can be taken from a subject, and the level of LDL cholesterol in the blood sample can be measured, for example, using the Friedewald Equation.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those with increased LDL cholesterol as well as those in which the increase in LDL cholesterol is to be prevented. Treatment also encompasses maintaining a lower level of LDL cholesterol. Thus, the whey protein hydrolysate can be used, for example, to lower LDL cholesterol, prevent an increase in LDL cholesterol, and to maintain a lowered LDL cholesterol level in a mammal.

A high level of LDL cholesterol has been associated with numerous indications. Thus, the whey protein hydrolysate will be useful in the treatment of any indication, the symptoms of which are directly or indirectly ameliorated by lowering or maintaining a low level of LDL cholesterol. For example, the whey protein hydrolysate will be useful to protect or treat the cardiovascular system, for example, to treat heart disease, atherosclerosis, stroke, angina, high blood pressure, and other circulatory and cardiovascular indications. The whey protein hydrolysate will also be able to treat insulin sensitivity, dementia, Alzheimer's disease, diabetes, or impotence.

Any ranges defined herein include the endpoints of the ranges.

The invention will now be described by the following non-limiting example.

Example 1

Study Participants:

Twelve untreated borderline hypertensives (blood pressure between

120/80-140/90) were recruited to participate in a preliminary dosing experiment (hereinafter "Dosing Evaluation", see below for description). For a pilot trial, thirty untreated borderline hypertensive, but otherwise healthy individuals were recruited.

All participants were selected based on the following criteria for inclusion and exclusion, which were designed to recruit adults who were generally healthy, unmedicated and borderline hypertensive.

Inclusion criteria:

- Blood Pressure: 120/80 or greater (with an upper limit of 140/90), and currently untreated
- Age >17 and <71 years
- Non-smokers
- Standard American diet
- Mostly sedentary at work and leisure

Exclusion criteria:

- Pregnant or lactating
- History or signs of excessive alcohol use (average more than 2 drinks per day)
- Dairy allergies or aversions
- Gastrointestinal disease
- Renal disease
- History of liver disease or dysfunction
- Elevated serum potassium >5 meq/L
- Any medication use that might interact with BioZate 1® hydrolyzed whey protein isolate: including diuretics, lithium, and sSRIs
- Unwillingness to comply with any part of the study protocol and/or failure to sign the consent form

Study design

Dosing Evaluation

This Dosing Evaluation was designed with the aid of prior *in vitro* and *in vivo* testing (see WO 01/85984) to determine appropriate dose for a subsequent Pilot Trial. The twelve participants selected included 6 men and 6 women. All

were healthy, mildly hypertensive, and unmedicated. Three treatment groups of two men and two women each were given one of three doses (5, 10, or 20 grams) of two Treatment Proteins (Test=BioZate 1® hydrolyzed whey protein isolate and Comparison= *Bi*PRO® whey protein isolate) and tested over two-24 hour periods. The *Bi*PRO® whey protein isolate was previously shown[4] to lack significant related activity and served as the comparison Treatment Protein for assessing activity of the BioZate 1® hydrolyzed whey protein isolate Test Treatment Protein. The order of the treatments was randomly assigned. Each participant reported in the early morning and was provided breakfast (meals were be matched for each person). One hour after breakfast, a first set of 4 blood pressure readings was taken using a standard protocol, after which each participant was asked to consume the appropriate amount of Treatment Protein (BioZate 1® hydrolyzed whey protein isolate or *Bi*PRO® whey protein isolate). The Treatment Protein for each dose was premixed into 4 to 8 ounces of pudding to improve the palatability of the Treatment Protein and improve compliance.

Blood pressure readings (4 sets using standard protocol) were taken each 1/2 hour for the first 4 hours and every hour throughout the rest of the day and evening, until just before bedtime. Checks for orthostatic hypotension were made at hour #1 and #3 after the consumption of the pudding containing Treatment Protein. The last blood pressure reading of the first day was taken just before bed. Blood pressure readings resumed the next day upon waking, until at least two sets of readings were taken. Each participant was given breakfast after the last two sets of readings. After at least a 24-hour washout, the participant reported back to repeat the protocol using the comparison Treatment Protein. In this way, each of the participants served as his or her own comparison.

The data, summarized in Figures 1-6, were analyzed using area-under-the curve method, and the appropriate dose and schedule (20 grams, once per day) were selected for the Pilot Trial. Dosing Evaluation participants were invited to participate in the Pilot Trial. There was a 2-week washout from the end of their participation in the Dosing Evaluation to randomization in the Pilot Trial.

Pilot Trial

A two-arm, controlled parallel group, double blind randomized Pilot Trial included two groups. Each participant was randomized to one of the following two treatment groups using a block randomization scheme:

Group 1 - Comparison group (to be administered 20g of *Bi*PRO® whey protein isolate placebo) and

Group 2 - Test group (to be administered 20g of BioZate 1® hydrolyzed whey protein isolate).

During a six-week treatment period, each participant consumed a daily chocolate Treatment Protein Beverage prepared from a Mix containing one of the following two Treatment Proteins:

(1) Test Treatment Protein included of 20 g of BioZate 1® hydrolyzed whey protein isolate, or

(2) Comparison Treatment Protein included of *Bi*PRO® whey protein isolate.

Each Treatment Protein was provided in a Treatment Protein Beverage Mix. The Beverage was prepared for serving by dissolving the Mix in 8 ounces of water.

Clinical Measurements

Clinical data was collected at screening (visit 1), and then weekly during the intervention (visits 2-8) and during a follow-up period (visits 9-12). Participants were asked to avoid: (1) strenuous exercise for 48 hours, (2) consuming alcohol and caffeine for one day and (3) consuming anything aside from water for 12 hours, before the clinic visits when blood was to be drawn.

Screening (visit #1). For each of the participants, a fasted blood sample was drawn, blood pressure was taken, and other information was verified. Screening lab tests included a kidney function panel and a measurement of serum potassium. Kidney dysfunction and elevated serum potassium were mandatory exclusion criteria.

Baseline (visit #2). This visit occurred within 1 week of the screening visit. Blood pressure was assessed using standardized methods. Body weight was checked at this and all visits. Blood samples were drawn for liver function

evaluation and complete white blood cell count. Participants were provided with a 7-day supply of the Test Group Treatment Protein Beverage Mix (with BioZate 1® hydrolyzed whey protein isolate) or the Comparison Group Treatment Protein Beverage Mix (with *Bi*PRO® whey protein isolate placebo). Participants were instructed to consume the Treatment Protein Beverage at the same time each day.

Weekly visits (visit #3-7). Participants returned every 7th day during the six-week duration of the study to get their next supply of Test Group Treatment Protein Beverage Mix or Comparison Group Treatment Protein Beverage Mix. Visits #3-8 occurred at one-week intervals starting after visit #2 and consisted of blood pressure assessment, side effect assessment, and body weight checks. In addition, blood samples were drawn at visit #5 to assess liver enzymes and total white blood cell count. If blood pressure exceeded 160/100 at any visit, the participant was withdrawn from the study and referred to his or her physician for more immediate aggressive treatment.

Final treatment visit (visit #8). Visit #8 was the last treatment study visit and occurred one week after visit #7. Blood pressure, side effects, and body weight were assessed as usual. Total lipids, liver function, and a complete white blood cell count were also assessed.

Biostatistical Analyses of Clinical Data

The primary analyses described differences between the treatments in systolic and diastolic blood pressure using paired t-tests and multiple measures regression. Secondary analyses described differences in side effects, lipids, white blood cell counts and markers of liver dysfunction using either multiple measures regression or paired and independent t-tests. In the data summaries presented in the Figures, the term "Active" (or A) refers to the Test Treatment Protein, BioZate 1® hydrolyzed whey protein isolate, and "Control" (or C) refers to the Comparison Treatment Protein, *Bi*PRO® whey protein isolate.

Summary of Clinical Testing and Conclusions

Dosing Evaluation

- Four subjects (2 men and 2 women) from a panel of 12 hypertensive

adults were randomized to receive one of three possible doses (5, 10, or 20 grams) of BioZate 1® hydrolyzed whey protein isolate (Test Treatment Protein) or *Bi*PRO® whey protein isolate (Comparison Treatment Protein).

- Each participant served as his own control, receiving the Comparison Treatment Protein after a 24-hour washout period. The order of the treatments was randomly assigned.
- Each treatment (BioZate 1® hydrolyzed whey protein isolate or *Bi*PRO® whey protein isolate) was administered one hour after a baseline blood pressure reading was taken early in the morning. Additional blood pressure readings were taken every 30 minutes (for the first four hours) and subsequently at one-hour intervals until bedtime and at least twice the next morning.
- Data were analyzed using area-under-the-curve analyses.
- Reductions in systolic blood pressure (SBP) occurred with all doses of hydrolyzed whey protein.
- The greatest SBP and diastolic blood pressure (DBP) reductions were experienced by subjects receiving either the 10 gram or 20 gram doses of BioZate 1® hydrolyzed whey protein isolate (Test Treatment Protein), with a greater mean area reduction for the 20 gram dose. The 20 gram dose was therefore selected for the Pilot Trial.

Pilot Trial

- 30 men and women (15 participants in each of two groups) were randomized to receive a 20g daily dose of either BioZate 1® hydrolyzed whey protein isolate (Test Treatment Group) or *Bi*PRO® whey protein isolate (Comparison Treatment Group). The doses were provided premixed in chocolate beverage powder, which was prepared for consumption by mixing it with 8 ounces of water.
- Participants were asked to take the prepared doses daily and to otherwise maintain their normal dietary and other lifestyle habits.
- On a weekly basis for 6 weeks, participants reported to the research clinic in the early morning for blood pressure checks.

- Four blood pressure (BP) readings were taken at each visit (using standard methodology) and were averaged.
- A significant (P=0.05) difference in BP reduction (BP effect) was found between the Test and Comparison Groups. BP effect, however, was also related to initial body weight and initial BP. Specifically, higher body mass index (BMI) resulted in a smaller BP change, and higher initial BP resulted in greater BP change. Neither of these BP effects was statistically significant between the Treatment and Comparison Groups. BP effect was not related to gender.
- Study-related side effects, blood lipids, liver function and white blood cell counts were also measured throughout the study. Compared to the Comparison group, subjects receiving BioZate 1® hydrolyzed whey protein isolate experienced a reduced SBP of 11mmHg and a reduced DBP of 7 mmHg. Study-related side effects did not differ by group.
- Liver function did not differ by group. There was a trend (P<0.09) toward significant low-density lipid cholesterol (LDL-C) reduction in the Test Treatment group compared to the Comparison Treatment group, with no significant change in high-density lipid cholesterol (HDL-C).
- Triglycerides were significantly increased in the Comparison group, much of the change being driven by one participant.
- There was no significant change in total cholesterol (Total-C) in either the Test or Comparison group.

Example 2

During phase I of the trial, 2 mean and 2 women were randomized to each of 3 groups: 5 g, 10 g and 20 g of BioZate 1® hydrolyzed whey protein isolate to be administered first thing in the morning. Each participant served as their own control. Blood pressures were assessed throughout the day, into the evening, and the next morning. Only 20g of BioZate resulted in a clinically significant drop in SBP and DBP, as assessed by area-under-the curve analyses, which persisted for 5-7 hours. Therefore, this dose was selected for the pilot trial.

During the pilot trial, blood pressure was assessed weekly for six weeks using a standardized protocol. The average of four readings was used in the analyses. Using repeated measure regression, SBP was reduced by 11 mmHg and DBP by 7 mmHg (unadjusted) after treatment with BioZate compared to *Bi*PRO® whey protein isolate. After adjustment for baseline differences in blood pressure and changes in body weight, differences in SBP between the groups was 10 mmHg and DBP was 6 mmHG. Body weight changed less than 1 Kg during the trial in each group and all lifestyle variables went unchanged from baseline. LDL-C and TC were significantly reduced in the treatment group. Side effects were assessed by questionnaire and evaluated possible blood pressure, ACE-inhibition and gastrointestinal-related events. No significant changes in any side effects occurred in either group. Additionally, no significant changes in renal or hepatic function occurred upon the addition of the treatment or control proteins.

Changes in serum lipids and other serum biochemical and clinical variables after treatment with BioZate 1® hydrolyzed whey protein isolate and *Bi*PRO® whey protein isolate

	BioZate			*Bi*PRO® whey protein isolate		
	Baseline	Mid-study	Post-Phase II	Baseline	Mid-study	Post-Phase II
LDL-C (mg/dL)‡	147.2±8.6	-	129.6±8.3*	145.7±8.8	-	143.2±8.8
HDL-C (mg/dL)	41.5±2.5	-	42.7±2.5	43.0±2.7	-	44.1±2.8
TC (mg/dL)‡	226.6±10.5	-	197.4±9.2*	222.9±10.7	-	219.3±10.1
TG (mg/dL)	151.9±15.0	-	146.3±14.6	168.6±16.6	-	157.9±16.1
BUN (mg/dL)	14.2±0.8	14.9±0.9	14.1±0.8	13.8±0.7	14.5±0.8	13.9±0.8
Creatinine (mg/dL)	1.05±0.04	1.09±0.05	1.06±0.04	0.99±0.03	1.05±0.04	1.06±0.04
GOT (U/L)	26+5	27±5	24±4	28±6	28±6	27±5
GPT (U/L)	20±6	21±6	22±5	21±6	24±6	22+6
Side Effects (210 possible score)	48.2±3.6	50.1±3.8	49.8±3.8	52.1±3.6	51.6±3.6	50.1±3.7
Body Weight (lbs)	178.5)±48	177.6±49	178.0±48	177.1±44	178.5±46	178.7±47

[1] Values are means ± SEM for all participants.
* Indicates statistical difference within a group from baseline at P<0.05.
‡ Indicates statistical differences between groups (change score) at P<0.05.

BioZate 1® hydrolyzed whey protein isolate		*Bi*PRO® whey protein isolate	
Phase II-baseline	Phase II-week 6	Phase II-baseline	Phase II-week 6
SBP 137 mmHg	127 mmHg*	135 mmHg	133 mmHg
DBP 84 mmHg	77 mmHg*	82 mmHg	80 mmHg

During phase III of the trial, participants originally randomized to the BioZate 1® hydrolyzed whey protein isolate group at 20 g/d had their dose elevated to 40 g/d, and those participants in the *Bi*PRO® whey protein isolate group were "crossed-over" to 20 g/d of BioZate 1® hydrolyzed whey protein isolate. This phase of the trial lasted for 12 weeks and blood pressures were assessed bi-weekly using the same methodology. As noted originally, in the BioZate 1® hydrolyzed whey protein isolate group, when "crossed-over" to the BioZate 1® hydrolyzed whey protein isolate group from Bi-Pro, LDL-C and TC was significantly reduced. In the original BioZate 1® hydrolyzed whey protein isolate group, LDL-C and TC were further reduced and WBC counts continued to be elevated. It is important to note that SE, hepatic function or renal function did not change at the elevated dose of BioZate 1® hydrolyzed whey protein isolate or when the control group was switched to the active treatment. Additional BP changes are noted below.

Changes in serum lipids and other serum biochemical and clinical variables after treatment with BioZate 1® hydrolyzed whey protein isolate and *Bi*PRO® whey protein isolate

		BioZate 1® hydrolyzed whey protein isolate (40 g)	Bi Pro	BioZate 1® hydrolyzed whey protein isolate (20 g)
	Baseline	Post-Phase III	Baseline	Post-Phase III
LDL-C (mg/dL)	147.2±8.6	123.5±8.1*	145.7±8.8	132.5±8.4*
HDL-C (mg/dL)	41.5±2.5	43.5±2.6	43.0±2.7	44.5±2.9
TC (mg/dL)	226.6±10.5	191.5±9.0*	222.9±10.7	204.4±9.5*
TG (mg/dL)	151.9±15.0	148.4±14.9	168.6±16.6	159.4±16.4
BUN (mg/dL)	14.2±0.8	13.8±0.7	13.8±0.7	13.3±0.7
Creatinine (mg/dL)	1.05±0.4	1.02±0.03	0.99±0.03	0.97±0.03
GOT (U/L)	26±5	26±5	28±6	31±6

GPT (U/L)	20±6	23±5	21±5	23±6
Side Effects (210 possible score)	48.2±3.6	52.7±3.9	52.1±3.6	49.5±3.5
Body Weight (lbs)	178.5±48	179.8±49	177.1±44	178.6±47

[1] Values are means ± SEM for all participants.
* Indicates statistical difference within a group at $P<0.05$.

BioZate 1® hydrolyzed whey protein isolate		*Bi*PRO® whey protein isolate to BioZate 1® hydrolyzed whey protein isolate	
Phase II-baseline	Phase III-week 12	Phase II-baseline	Phase III-week 12
SBP 137 mmHg	120 mmHg*	135 mmHg	121 mmHg*
DBP 84 mmHg	73 mmHg*	82 mmHg	74 mmHg*

All participants completed the 4-week follow-up phase, which consisted of weekly blood pressure measurements, body weights, and side effect questionnaires. Recall that all participants originally randomized to the BioZate 1® hydrolyzed whey protein isolate group completed phase III of the study on 40 grams of BioZate 1® hydrolyzed whey protein isolate and those originally randomized to the *Bi*PRO® whey protein isolate group completed the study on 20 grams of BioZate 1® hydrolyzed whey protein isolate. In both cases, the anti-hypertensive effects of BioZate 1® hydrolyzed whey protein isolate were "washed-out" completely within the first week of the follow-up phase (See mean BP data below). During the follow-up phase, all dietary and lifestyle variables remained unchanged.

Changes in serum lipids and other serum biochemical and clinical variables after treatment with BioZate 1® hydrolyzed whey protein isolate and *Bi*PRO® whey protein isolate

	BioZate 1® hydrolyzed whey protein isolate		*Bi*PRO® whey protein isolate	
	Baseline	Follow-up	Baseline	Follow-up
LDL-C (mg/dL)	147.2±8.6	141.5±8.3	145.7±8.8	143.6±8.5
HDL-C (mg/dL)	41.5±2.5	42.2±2.3	43.0±2.7	44.7±2.9
TC (mg/dL)	226.6±10.5)	221.5±10.3	222.9±10.7	226.7±10.8
TG (mg/dL)	151.9±15.0	145.1±14.5	168.6±16.6	157.1±16.1
Side Effects (210 possible score)	48.2±3.6	47.5±3.5	52.1±3.6	50.2±3.7
Body Weight (lbs)	178.5±48	180.1±50	177.1±44	179.1±47

[1] Values are means ± SEM for all participants and means did not differ significantly.

BioZate 1® hydrolyzed whey protein isolate		
Phase III	week 1 follow-up	week 4 follow-up
SBP 120 mmHg	138 mmHg	139 mmHg
DBP 73 mmHg	85 mmHg	87 mmHg
*Bi*PRO® whey protein isolate		
Phase III	week 1 follow-up	week 4 follow-up
SBP 121 mmHg	134 mmHg	135 mmHg
DBP 74 mmHg	85 mmHg	88 mmHg

The present invention also provides a method to produce any of the beneficial effects disclosed hereinabove by administering the hydrolysate to a mammal, as well as the use of the hydrolysate in the manufacture of a medicament or an edible product useful to produce such beneficial effects.

Example 3

The ACE-inhibitory peptides in BioZate 1® hydrolyzed whey protein isolate were isolated and characterized.

Semi-preparative fractionation of hydrolyzed whey protein isolate (BioZate 1® hydrolyzed whey protein isolate) is required for isolation and identification of peptide fractions. These isolated peptide fractions were later evaluated for their ACE-inhibitory activity.

*Bi*PRO® whey protein isolate was hydrolyzed to produce BioZate 1® hydrolyzed whey protein isolate. The enzymatic reaction was stopped by ultrafiltration (UF) using a 30 kilodalton polyethersulfone membrane instead of pasteurization (HTST) treatment; the permeate was further freeze-dried, and is referred to as "UF-BioZate".

UF-BioZate (2 mL of 20% TS solution) was fractionated using a semi-preparative anionic exchange chromatography HPLC system (Gold Prep 60, Beckman, CA) equipped with a semi-preparative cell model 166, pumps model 126, detector UV/VIS model 166 adjusted at 214 nm, manual injector (2 mL) Rheodyne model 7725 (Cotati, CA), and Gold Nouveau software. The hydrolysate was fractionated using an acid-tolerant Luna 10 μ C18 column (250 x 21.2 mm) from Phenomenex (Torrance, Ca).

For elution of peptide material, mobile phase-A (5 mM HCl) and mobile phase-B (60% ACN/5 mM HCl) were circulated in the column at a flow rate of 20 mL/min and according to the following gradient:

0-15% B from 0-20 min;
15-25% B from 20-30 min;
25-35% B from 30-45 min;
35-60% B from 45-60 min;
60-100% B from 60-65 min;
100% B from 65-70 min;
100-0% B from 70-75 min.

During elution, eight peptide fractions (F1-F8) were collected as shown in Figure 19. Approximately 1.1-2.5 liters of eluate per fraction were collected. Fractions that contained large amount of salts were further desalted by using a semi-prep C 18 column. After evaporation of solvent (ACN/TFA) used for the elution of peptide material with C18 column, protein content of the peptide fractions was measured by Leco/Dumas method. All the fractions collected were freeze-dried.

The peptide composition of each fraction (F1–F8) was evaluated by reverse phase high performance liquid chromatography (RP-HPLC C18). The ACE-inhibitory activity of these fractions was also evaluated. The presence of

specific peptide sequences in these fractions (F1–F8) was established by comparing the retention times of synthesized pure peptide sequences with those of specific peaks obtained from the fractions. The ACE-inhibitory values (IC50 values) of these synthetic peptide sequences were compared to those of the fractions to further substantiate the presence of such ACE-inhibitory peptides in BioZate 1® hydrolyzed whey protein isolate. The values obtained from the analysis are shown below.

Protein and IC_{50} values of peptide fractions (F1–F8) collected from semi-prep RP-HPLC and different peptides obtained from chemical synthesis.

Fractions	Volume (L)	Protein content[1] (%)	IC_{50} ± SD (mg powder/mL)	IC_{50} (mg protein/mL)
F1	1.2	69.27	1.469±0.004	1.108
F2	1.7	64.43	1.029±0.001	0.663
F3	1.4	63.84	0.610±0.002	0.389
F4	1.2	63.67	2.521±0.108	1.605
F5	1.1	58.93	0.480±0.007	0.283
F6	1.5	61.98	1.014±0.020	0.629
F7	1.1	50.07	4.306±0.329	2.156
F8	2.5	39.31	2.616±0.104	1.028
BioZate 1® hydrolyzed whey protein isolate	-	92.5	1.400±0.042	1.295
UF-BioZate	-	93.7	0.823±0.024	0.771
β-LG 142-148	Synthetic	NA[2]	0.253±0.008	-
β-LG 9-14	Synthetic	NA[2]	NS[3]	-
β-LG 84-91	Synthetic	NA[2]	NS[3]	-
β-LG 125-135	Synthetic	NA[2]	NS[3]	-
β-LG 1-8	Synthetic	NA[2]	NS[3]	-
β-LG 15-20	Synthetic	NA[2]	0.220±0.001[4]	-
β-LG 71-75	Synthetic	NA[2]	NS[3]	-
β-LG 78-83	Synthetic	NA[2]	0.460±0.027[4]	-
β-LG 92-100	Synthetic	NA[2]	NS[3]	-

[1] Protein content was determined by Leco analysis;

[2] NA=Not available;

[3] NS=Result not significant;

[4] triplicate analysis.

It appears that the UF permeate of the hydrolyzed whey protein isolate (UF-BioZate) has increased ACE-inhibitory activity because of the elimination of majority of the non-hydrolyzed protein and possible concentration of the active peptides in this material. These effects explain the higher anti-ACE activity of the UF-BioZate compared to BioZate 1® hydrolyzed whey protein isolate. It is also obvious from these results that the anti-ACE activity is found in some of the fractions but not in others, indicating that the specific peptides responsible for ACE inhibition are eluted only in some of the fractions. Specifically, fractions F3 and F5 show a markedly high ACE inhibitory activity.

The results show that peptides β-LG 142-148, β-LG 15-20, and β-LG 78-83 are potent ACE inhibitory peptides. The presence of the peptide β-LG 142-148 and β-LG 15-20 has been confirmed in fraction F3 by comparing the retention times, shown in Figure 20A. Presence of the peptide β-LG 15-20 has been confirmed in fraction F3 by comparing the retention times, shown in Figure 21B.

Thus, ACE-inhibitory peptides are present in BioZate 1® hydrolyzed whey protein isolate.

All publications, patents and patent applications referred to herein are incorporated by reference. While in the foregoing specification this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein may be varied considerably without departing from the basic principles of the invention.

What is claimed is:

1. The use of a whey protein hydrolysate in the manufacture of a medicament useful to lower low-density lipoprotein (LDL) cholesterol in a mammal.

2. The use according to claim 1, wherein the medicament is also useful to treat hypertension in a mammal.

3. The use of a whey protein hydrolysate in the manufacture of an edible product useful to lower low-density lipoprotein (LDL) cholesterol in a mammal.

4. The use according to claim 3, wherein the product is also useful to treat hypertension in a mammal.

5. The use according to claim 3, wherein the edible product is a food product.

6. The use according to claim 3, wherein the food product is beverage product, a dessert product, a confectionary product, a baked product, a dairy product, a cereal, a bread, a muffin, a cake, a health food bar or a snack bar.

7. The use according to any one of claims 1-6, wherein the whey protein hydrolysate is prepared according to the process of preparing a solution comprising a whey protein sample and at least one proteolytic enzyme, and holding the solution under conditions effective to partially hydrolyze the whey protein to provide a hydrolysate having LDL cholesterol lowering properties.

8. The use according to claim 7, wherein the whey protein sample is a whey protein fraction.

9. The use according to claim 7, wherein the whey protein sample is a whey protein concentrate.

10. The use according to claim 7, wherein the whey protein sample is a whey protein isolate.

11. The use according to any one of claims 7-10, wherein the whey protein sample is prepared by ion-exchange processing.

12. The use according to any one of claims 7-11, wherein the whey protein sample has a Protein Digestibility Corrected Amino Acid Score of about 1.14.

13. The use according to any one of claims 7-12, wherein the whey protein sample has an ash content of less than about 3%.

14. The use according to any one of claims 7-13, wherein the whey protein sample has a mineral content of calcium in the range of from about 15-20 meq/kg.

15. The use according to any one of claims 7-14, wherein the whey protein sample has a mineral content of magnesium of about 1 meq/kg.

16. The use according to any one of claims 7-15, wherein the whey protein sample has a fat and lactose content of less than about 1%.

17. The use according to any one of claims 7-16, wherein the whey protein sample contains at least about 55% β-lactoglobulin.

18. The use according to any one of claims 7-17, wherein the whey protein sample has a protein content of at least 35%.

19. The use according to any one of claims 7-18, wherein the whey protein sample has a protein content that is within the range of 0 to 25% of 97.8%.

20. The use according to any one of claims 7-19, wherein the whey protein

sample has a protein content that is within the range of 5-10% of 97.8%.

21. The use according to any one of claims 7-20, wherein the whey protein sample has a protein content that varies less than 5% from 97.8%.

22. The use according to any one of claims 7-21, wherein the whey protein sample has a protein content that is 97.8 ± 0.4%.

23. The use according to any one of claims 7-22, wherein the whey protein sample is characterized as follows:

Analysis	Range
Moisture (%)	4.9 ± 0.1
Protein, dry basis (%)	97.8 ± 0.4
Fat (%)	0.3 ± 0.1
Ash (%)	2.0 ± 0.3
Lactose (%)	<0.5
pH	7.2 ± 0.1

24. The use according to any one of claims 7-23, wherein the solution comprises the proteolytic enzyme trypsin.

25. The use according to any one of claims 7-24, wherein the solution is hydrolyzed at a pH within the range of from 8.0-8.5.

26. The use according to any one of claims 7-25, wherein the whey protein sample is solubilized at about 20% w/v.

27. The use according to any one of claims 7-26, wherein the solution is held at a temperature within the range of from 40°-50°C.

28. The use according to any one of claims 7-27, wherein the hydrolysis of the whey protein sample is stopped by heat treatment.

29. The use according to any one of claims 7-27, wherein the hydrolysis of the whey protein sample is stopped by ultrafiltration.

30. The use according to any one of claims 7-29, further comprising drying the hydrolysate following hydrolysis.

31. The use according to any one of claims 7-29, further comprising spray drying the hydrolysate following hydrolysis.

32. The use according to any one of claims 7-29, further comprising freeze drying the hydrolysate following hydrolysis.

33. The use according to any one of claims 7-32, wherein the whey protein hydrolysate is characterized by a degree of hydrolysis within the range of from about 5.5-6.5%.

34. The use according to any one of claims 7-33, wherein the whey protein hydrolysate is characterized by the molecular weight profile:

Molecular Weight Profile (Daltons)	Peptides
Greater than 10,000	30 – 45%
5,000 – 10,000	7 – 12%
2,000 – 5,000	15 – 25%
Less than 2,000	30 – 45%

35. The use according to any one of claims 7-34, wherein the whey protein hydrolysate is characterized as follows:

Analysis	Range
Moisture (%)	5.2 ± 0.3
Protein, dry basis (%)	91.0 ± 0.5
Amino Nitrogen (AN) (%)	1.8 ± 0.3
AN/TN (%)	12.8 ± 1.0
Degree of Hydrolysis (%)	5.5 ± 1.5
Fat (%)	<1.0
Ash (%)	5.5 ± 0.3
Lactose (%)	<1.0
pH	8.0 ± 0.2

36. An article, comprising:

a) an edible product comprising a whey protein hydrolysate; and

b) packaging material, wherein the packaging material is marked to indicate that the product gives the benefit of lowering cholesterol.

37. The article according to claim 36, wherein the packaging material is marked to indicate that the product lowers low-density lipoprotein (LDL) cholesterol.

38. The article according to any one of claims 36-37, wherein the packaging material is marked to indicate that the whey protein hydrolysate gives the benefit of lowering cholesterol.

39. The article according to any one of claims 36-38, wherein the packaging material is marked to indicate that the product also gives the benefit of treating hypertension.

40. The article according to any one of claims 36-39, wherein the edible product is a food product.

41. The article according to claim 40, wherein the food product is beverage product, a dessert product, a confectionary product, a baked product, a dairy product, a cereal, a bread, a muffin, a cake, a health food bar or a snack bar.

42. The article according to any one of claims 36-41, wherein the whey protein hydrolysate is prepared according to the process of preparing a solution comprising a whey protein sample and at least one proteolytic enzyme, and holding the solution under conditions effective to partially hydrolyze the whey protein to provide a hydrolysate having LDL cholesterol lowering properties.

43. The article according to claim 42, wherein the whey protein sample is a whey protein fraction.

44. The article according to claim 42, wherein the whey protein sample is a whey protein concentrate.

45. The article according to claim 42, wherein the whey protein sample is a whey protein isolate.

46. The article according to any one of claims 42-45, wherein the whey protein sample is prepared by ion-exchange processing.

47. The article according to any one of claims 42-46, wherein the whey protein sample has a Protein Digestibility Corrected Amino Acid Score of about 1.14.

48. The article according to any one of claims 42-47, wherein the whey protein sample has an ash content of less than about 3%.

49. The article according to any one of claims 42-48, wherein the whey protein sample has a mineral content of calcium in the range of from about 15-20 meq/kg.

50. The article according to any one of claims 42-49, wherein the whey protein sample has a mineral content of magnesium of less than about 1 meq/kg.

51. The article according to any one of claims 42-50, wherein the whey protein sample has a fat and lactose content of less than about 1%.

52. The article according to any one of claims 42-51, wherein the whey protein sample contains at least about 55% β-lactoglobulin.

53. The article according to any one of claims 42-52, wherein the whey protein sample has a protein content of at least 35%.

54. The article according to any one of claims 42-53, wherein the whey protein sample has a protein content that is within the range of 0 to 25% of 97.8%.

55. The article according to any one of claims 42-54, wherein the whey protein sample has a protein content that is within the range of 5-10% of 97.8%.

56. The article according to any one of claims 42-55, wherein the whey protein sample has a protein content that varies less than 5% from 97.8%.

57. The article according to any one of claims 42-56, wherein the whey protein sample has a protein content that is 97.8 ± 0.4%.

58. The article according to any one of claims 42-57, wherein the whey protein sample is characterized as follows:

Analysis	Typical
Moisture (%)	4.9 ± 0.1
Protein, dry basis (%)	97.8 ± 0.4
Fat (%)	0.3 ± 0.1
Ash (%)	2.0 ± 0.3
Lactose (%)	<0.5
pH	7.2 ± 0.1

59. The article according to any one of claims 42-58, wherein the solution comprises the proteolytic enzyme trypsin.

60. The article according to any one of claims 42-59, wherein the solution is hydrolyzed at a pH within the range of from 8.0-8.5.

61. The article according to any one of claims 42-60, wherein the whey protein sample is solubilized at about 20% w/v.

62. The article according to any one of claims 42-61, wherein the solution is held at a temperature within the range of from 40°-50°C.

63. The article according to any one of claims 42-62, wherein the hydrolysis of the whey protein sample is stopped by heat treatment.

64. The article according to any one of claims 42-62, wherein the hydrolysis of the whey protein sample is stopped by ultrafiltration.

65. The article according to any one of claims 42-64, further comprising drying the hydrolysate following hydrolysis.

66. The article according to any one of claims 42-64, further comprising spray drying the hydrolysate following hydrolysis.

67. The article according to any one of claims 42-64, further comprising freeze drying the hydrolysate following hydrolysis.

68. The article according to any one of claims 42-67, wherein the whey protein hydrolysate is characterized by a degree of hydrolysis within the range of from about 5.5-6.5%.

69. The article according to any one of claims 42-68, wherein the whey protein hydrolysate is characterized by the molecular weight profile:

Molecular Weight Profile (Daltons)	Peptides
Greater than 10,000	30 – 45%
5,000 – 10,000	7 – 12%
2,000 – 5,000	15 – 25%
Less than 2,000	30 – 45%

70. The article according to any one of claims 42-69, wherein the whey protein hydrolysate is characterized as follows:

Analysis	Typical
Moisture (%)	5.2 ± 0.3
Protein, dry basis (%)	91.0 ± 0.5
Amino Nitrogen (AN) (%)	1.8 ± 0.3
AN/TN (%)	12.8 ± 1.0
Degree of Hydrolysis (%)	5.5 ± 1.5
Fat (%)	<1.0
Ash (%)	5.5 ± 0.3
Lactose (%)	<1.0
pH	8.0 ± 0.2

71. A treatment for a mammal to lower low-density lipoprotein (LDL) cholesterol, comprising feeding to the mammal a whey protein hydrolysate in amounts and at intervals effective to lower LDL cholesterol.

72. The treatment of claim 71, wherein the feeding of the hydrolysate also treats hypertension.

73. The treatment according to any one of claims 71-72, wherein the whey protein hydrolysate is prepared according to the process of preparing a solution comprising a whey protein sample and at least one proteolytic enzyme, and holding the solution under conditions effective to partially hydrolyze the whey protein to provide a hydrolysate having LDL cholesterol lowering properties.

74. The treatment according to claim 73, wherein the whey protein sample is a whey protein fraction.

75. The treatment according to claim 73, wherein the whey protein sample is

a whey protein concentrate.

76. The treatment according to claim 73, wherein the whey protein sample is a whey protein isolate.

77. The treatment according to any one of claims 73-76, wherein the whey protein sample is prepared by ion-exchange processing.

78. The treatment according to any one of claims 73-77, wherein the whey protein sample has a Protein Digestibility Corrected Amino Acid Score of about 1.14.

79. The treatment according to any one of claims 73-78, wherein the whey protein sample has an ash content of less than about 3%.

80. The treatment according to any one of claims 73-79, wherein the whey protein sample has a mineral content of calcium in the range of from about 15-20 meq/kg.

81. The treatment according to any one of claims 73-80, wherein the whey protein sample has a mineral content of magnesium of less than about 1 meq/kg.

82. The treatment according to any one of claims 73-81, wherein the whey protein sample has a fat and lactose content of less than about 1%.

83. The treatment according to any one of claims 73-82, wherein the whey protein sample contains at least about 55% β-lactoglobulin.

84. The treatment according to any one of claims 73-83, wherein the whey protein sample has a protein content of at least 35%.

85. The treatment according to any one of claims 73-84, wherein the whey protein sample has a protein content that is within the range of 0 to 25% of

97.8%.

86. The treatment according to any one of claims 73-85, wherein the whey protein sample has a protein content that is within the range of 5-10% of 97.8%.

87. The use according to any one of claims 73-86, wherein the whey protein sample has a protein content that varies less than 5% from 97.8%.

88. The treatment according to any one of claims 73-87, wherein the whey protein sample has a protein content that is 97.8 ± 0.4%.

89. The treatment according to any one of claims 73-88, wherein the whey protein sample is characterized as follows:

Analysis	Typical
Moisture (%)	4.9 ± 0.1
Protein, dry basis (%)	97.8 ± 0.4
Fat (%)	0.3 ± 0.1
Ash (%)	2.0 ± 0.3
Lactose (%)	<0.5
pH	7.2 ± 0.1

.

90. The treatment according to any one of claims 73-89, wherein the solution comprises the proteolytic enzyme trypsin.

91. The treatment according to any one of claims 73-90, wherein the solution is hydrolyzed at a pH within the range of from 8.0-8.5.

92. The treatment according to any one of claims 73-91, wherein the whey protein sample is solubilized at about 20% w/v.

93. The treatment according to any one of claims 73-92, wherein the solution is held at a temperature within the range of from 40°-50°C.

94. The treatment according to any one of claims 73-93, wherein the hydrolysis of the whey protein sample is stopped by heat treatment.

95. The treatment according to any one of claims 73-93, wherein the hydrolysis of the whey protein sample is stopped by ultrafiltration.

96. The treatment according to any one of claims 73-95, further comprising drying the hydrolysate following hydrolysis.

97. The treatment according to any one of claims 73-96, further comprising spray drying the hydrolysate following hydrolysis.

98. The treatment according to any one of claims 73-96, further comprising freeze drying the hydrolysate following hydrolysis.

99. The treatment according to any one of claims 73-98, wherein the whey protein hydrolysate is characterized by a degree of hydrolysis within the range of from about 5.5-6.5%.

100. The treatment according to any one of claims 73-99, wherein the whey protein hydrolysate is characterized by the molecular weight profile:

Molecular Weight Profile (Daltons)	Peptides
Greater than 10,000	30 – 45%
5,000 – 10,000	7 – 12%
2,000 – 5,000	15 – 25%
Less than 2,000	30 – 45%

101. The treatment according to any one of claims 73-100, wherein the whey

protein hydrolysate is characterized as follows:

Analysis	Typical
Moisture (%)	5.2 ± 0.3
Protein, dry basis (%)	91.0 ± 0.5
Amino Nitrogen (AN) (%)	1.8 ± 0.3
AN/TN (%)	12.8 ± 1.0
Degree of Hydrolysis (%)	5.5 ± 1.5
Fat (%)	<1.0
Ash (%)	5.5 ± 0.3
Lactose (%)	<1.0
pH	8.0 ± 0.2

102. A method to lower low-density lipoprotein (LDL) cholesterol in a mammal in need of such treatment, comprising feeding to the mammal a whey protein hydrolysate in amounts and at intervals effective to lower LDL cholesterol.

103. The method of claim 102, wherein the feeding of the hydrolysate also treats hypertension.

104. The method according to any one of claims 102-103, wherein the whey protein hydrolysate is prepared according to the process of preparing a solution comprising a whey protein sample and at least one proteolytic enzyme, and holding the solution under conditions effective to partially hydrolyze the whey protein to provide a hydrolysate having LDL cholesterol lowering properties.

105. The method according to claim 104, wherein the whey protein sample is a whey protein fraction.

106. The method according to claim 104, wherein the whey protein sample is

a whey protein concentrate.

107. The method according to claim 104, wherein the whey protein sample is a whey protein isolate.

108. The method according to any one of claims 104-107, wherein the whey protein sample is prepared by ion-exchange processing.

109. The method according to any one of claims 104-108, wherein the whey protein sample has a Protein Digestibility Corrected Amino Acid Score of about 1.14.

110. The method according to any one of claims 104-109, wherein the whey protein sample has an ash content of less than about 3%.

111. The method according to any one of claims 104-110, wherein the whey protein sample has a mineral content of calcium in the range of from about 15-20 meq/kg.

112. The method according to any one of claims 104-111, wherein the whey protein sample has a mineral content of magnesium of less than about 1 meq/kg.

113. The method according to any one of claims 104-112, wherein the whey protein sample has a fat and lactose content of less than about 1%.

114. The method according to any one of claims 104-113, wherein the whey protein sample contains at least about 55% β-lactoglobulin.

115. The method according to any one of claims 104-114, wherein the whey protein sample has a protein content of at least 35%.

116. The method according to any one of claims 104-115, wherein the whey protein sample has a protein content that is within the range of 0 to 25% of

97.8%.

117. The method according to any one of claims 104-116, wherein the whey protein sample has a protein content that is within the range of 5-10% of 97.8%.

118. The method according to any one of claims 104-117, wherein the whey protein sample has a protein content that varies less than 5% from 97.8%.

119. The method according to any one of claims 104-118, wherein the whey protein sample has a protein content that is 97.8 ± 0.4%.

120. The method according to any one of claims 104-119, wherein the whey protein sample is characterized as follows:

Analysis	Typical
Moisture (%)	4.9 ± 0.1
Protein, dry basis (%)	97.8 ± 0.4
Fat (%)	0.3 ± 0.1
Ash (%)	2.0 ± 0.3
Lactose (%)	<0.5
pH	7.2 ± 0.1

.

121. The method according to any one of claims 104-120, wherein the solution comprises the proteolytic enzyme trypsin.

122. The method according to any one of claims 104-121, wherein the solution is hydrolyzed at a pH within the range of from 8.0-8.5.

123. The method according to any one of claims 104-122, wherein the whey protein sample is solubilized at about 20% w/v.

124. The method according to any one of claims 104-123, wherein the solution is held at a temperature within the range of from 40°-50°C.

125. The method according to any one of claims 104-124, wherein the hydrolysis of the whey protein sample is stopped by heat treatment.

126. The method according to any one of claims 104-124, wherein the hydrolysis of the whey protein sample is stopped by ultrafiltration.

127. The method according to any one of claims 104-126, further comprising drying the hydrolysate following hydrolysis.

128. The method according to any one of claims 104-127, further comprising spray drying the hydrolysate following hydrolysis.

129. The method according to any one of claims 104-127, further comprising freeze drying the hydrolysate following hydrolysis.

130. The method according to any one of claims 104-129, wherein the whey protein hydrolysate is characterized by a degree of hydrolysis within the range of from about 5.5-6.5%.

131. The method according to any one of claims 104-130, wherein the whey protein hydrolysate is characterized by the molecular weight profile:

Molecular Weight Profile (Daltons)	Peptides
Greater than 10,000	30 – 45%
5,000 – 10,000	7 – 12%
2,000 – 5,000	15 – 25%
Less than 2,000	30 – 45%

132. The method according to any one of claims 104-131, wherein the whey protein hydrolysate is characterized as follows:

Analysis	Typical
Moisture (%)	5.2 ± 0.3
Protein, dry basis (%)	91.0 ± 0.5
Amino Nitrogen (AN) (%)	1.8 ± 0.3
AN/TN (%)	12.8 ± 1.0
Degree of Hydrolysis (%)	5.5 ± 1.5
Fat (%)	<1.0
Ash (%)	5.5 ± 0.3
Lactose (%)	<1.0
pH	8.0 ± 0.2

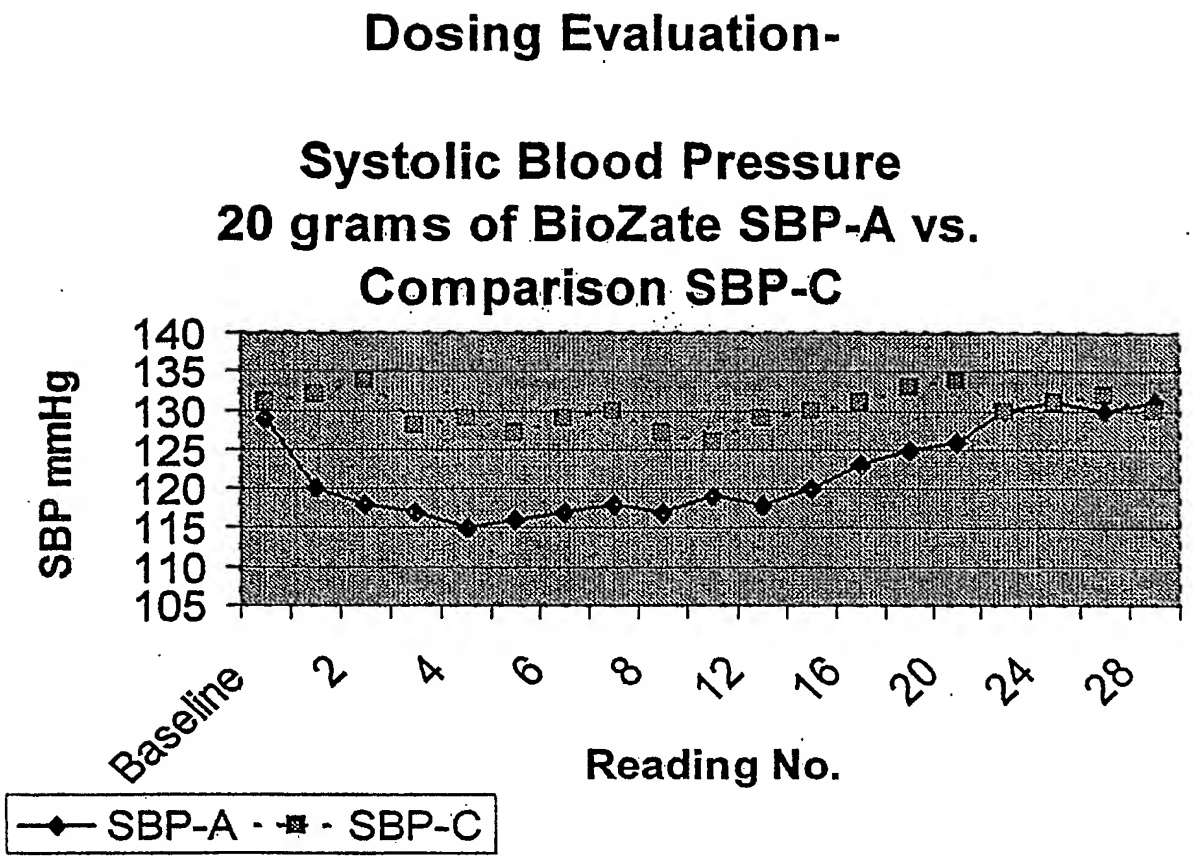
Dosing Evaluation-
Systolic Blood Pressure
20 grams of BioZate SBP-A vs.
Comparison SBP-C
SBP mmHg
140
135
130
125
120
115
110
105
Baseline
2
4
6
8
12
16
20
24
28
Reading No.
SBP-A
SBP-C

FIG. 1

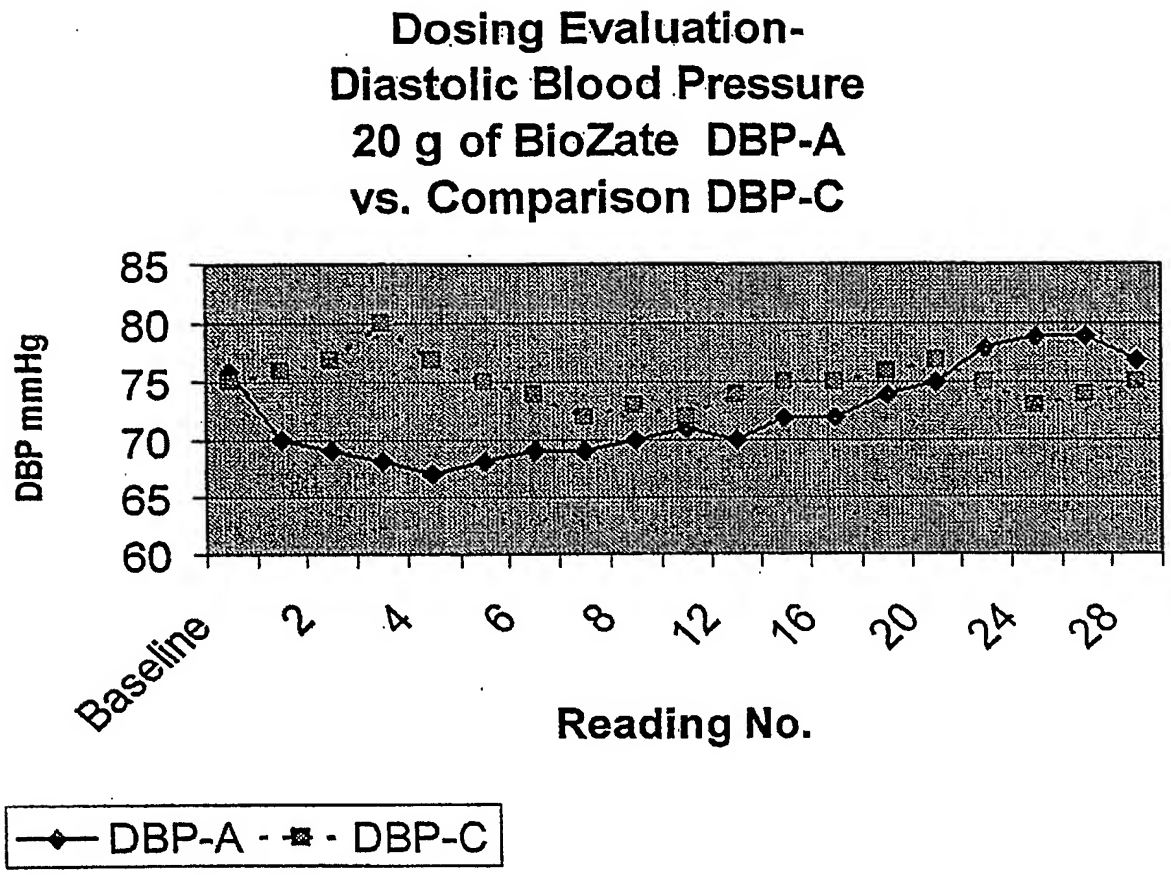
Dosing Evaluation-
Diastolic Blood Pressure
20 g of BioZate DBP-A
vs. Comparison DBP-C
DBP mmHg
85
80
75
70
65
60
Baseline
2
4
6
8
12
16
20
24
28
Reading No.
DBP-A
DBP-C

FIG. 2

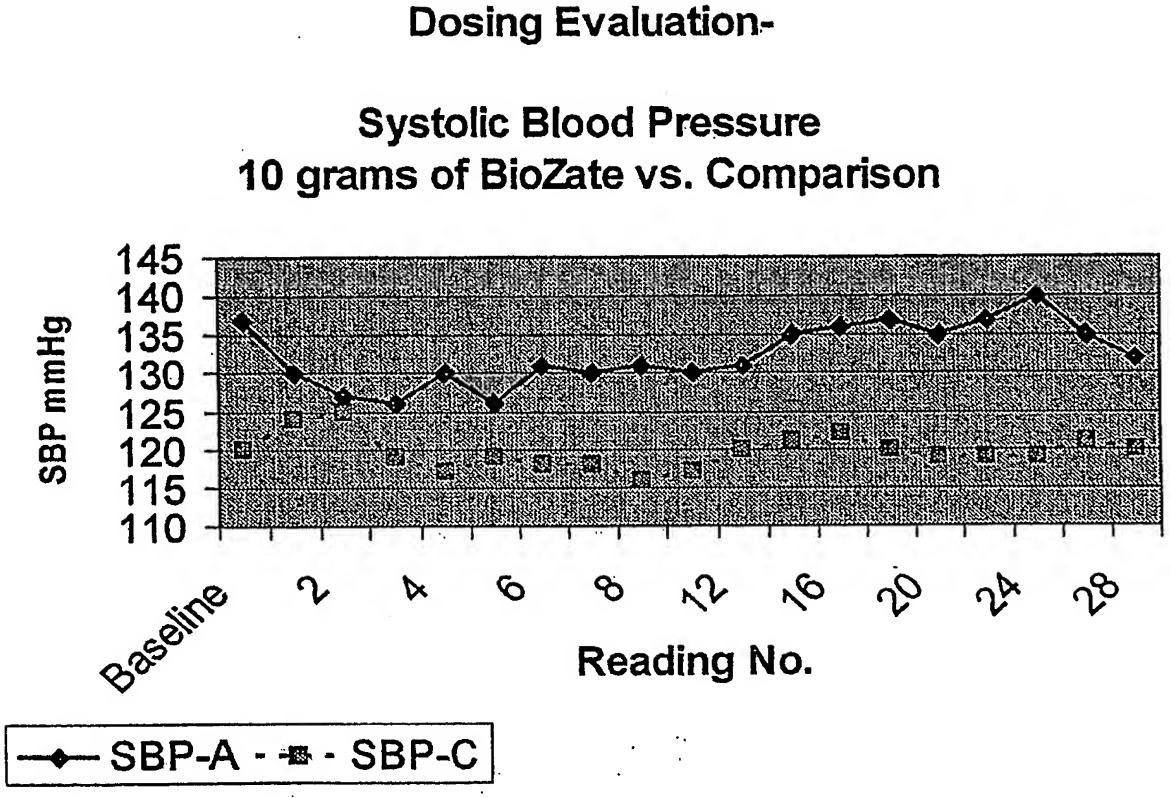
Dosing Evaluation-
Systolic Blood Pressure
10 grams of BioZate vs. Comparison
SBP mmHg
145
140
135
130
125
120
115
110
Baseline
2
4
6
8
12
16
20
24
28
Reading No.
SBP-A
SBP-C

FIG. 3

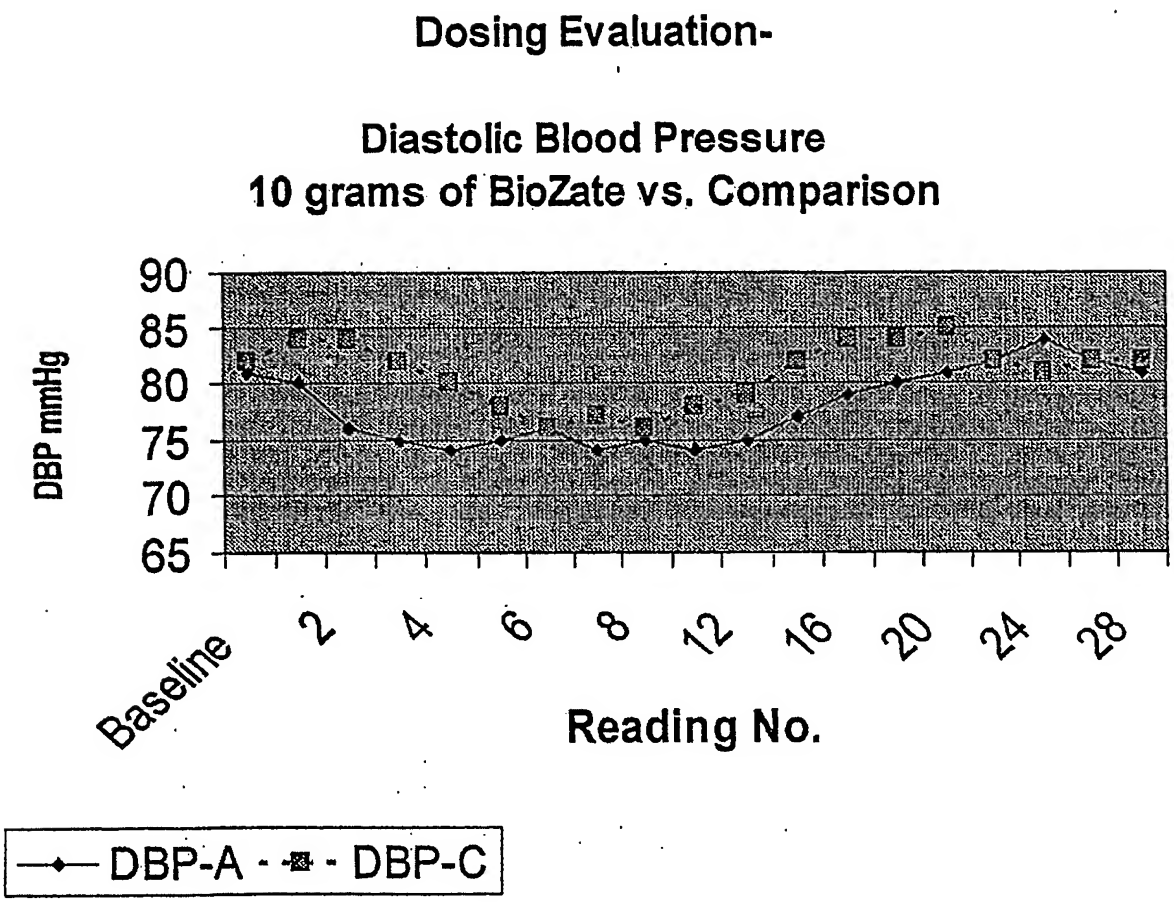
Dosing Evaluation-
Diastolic Blood Pressure
10 grams of BioZate vs. Comparison
DBP mmHg
90
85
80
75
70
65
Baseline
2
4
6
8
12
16
20
24
28
Reading No.
DBP-A
DBP-C

FIG. 4

Dosing Evaluation-

Systolic Blood Pressure
5 grams of BioZate vs. Comparison

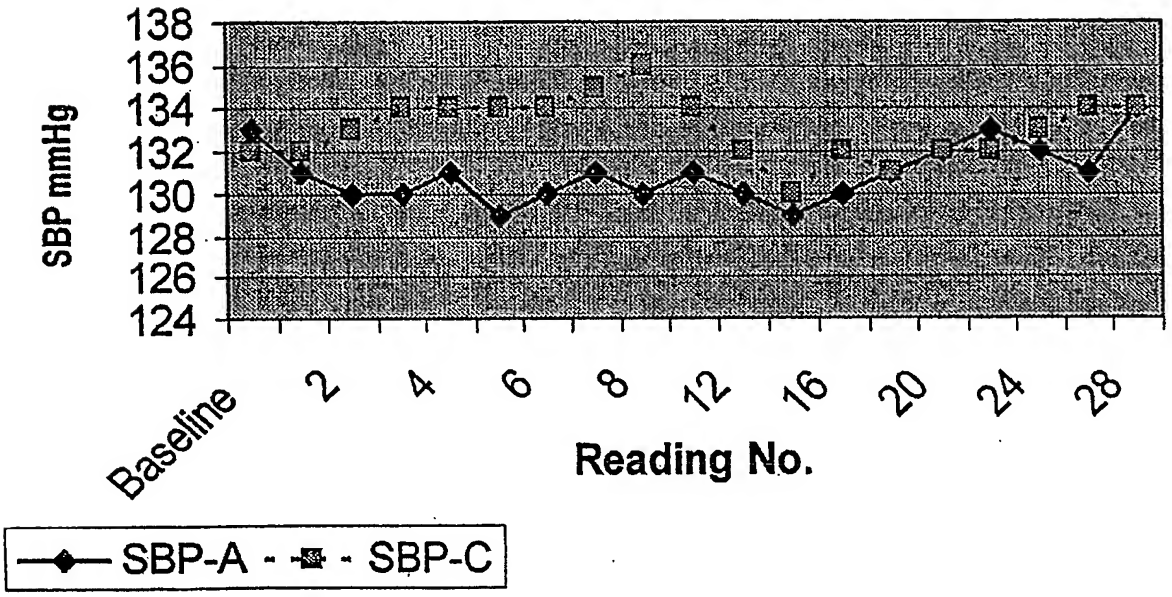

FIG. 5

Dosing Evaluation-

Diastolic Blood Pressure
5 grams of BioZate vs. Comparison

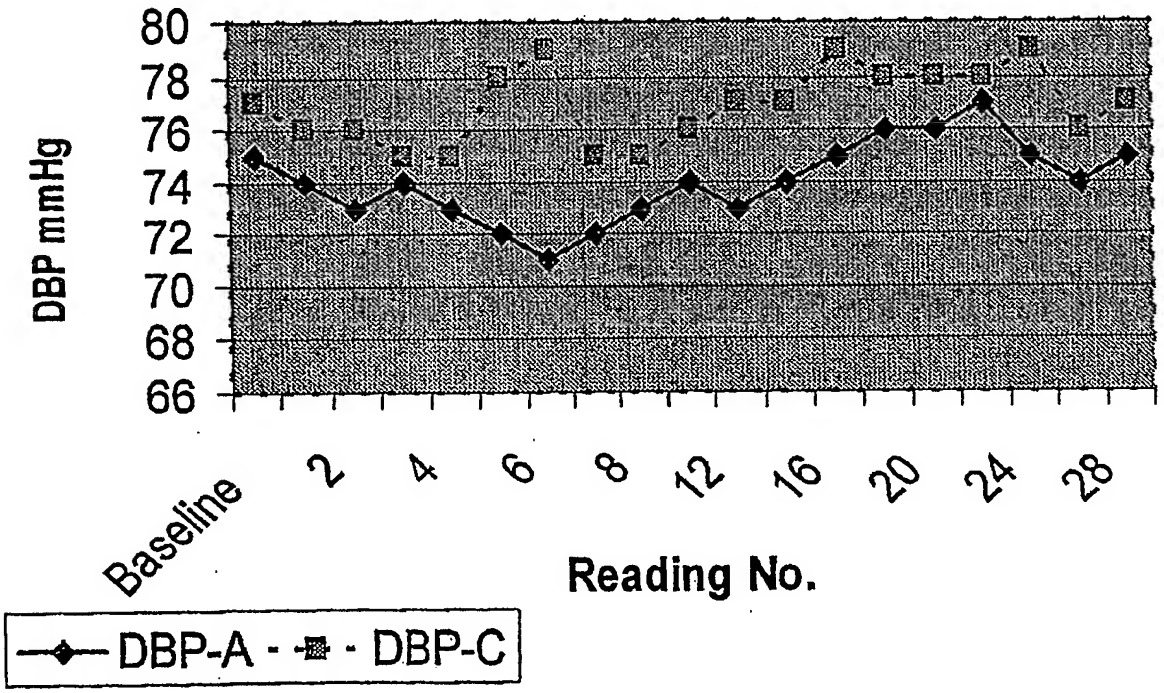

FIG. 6

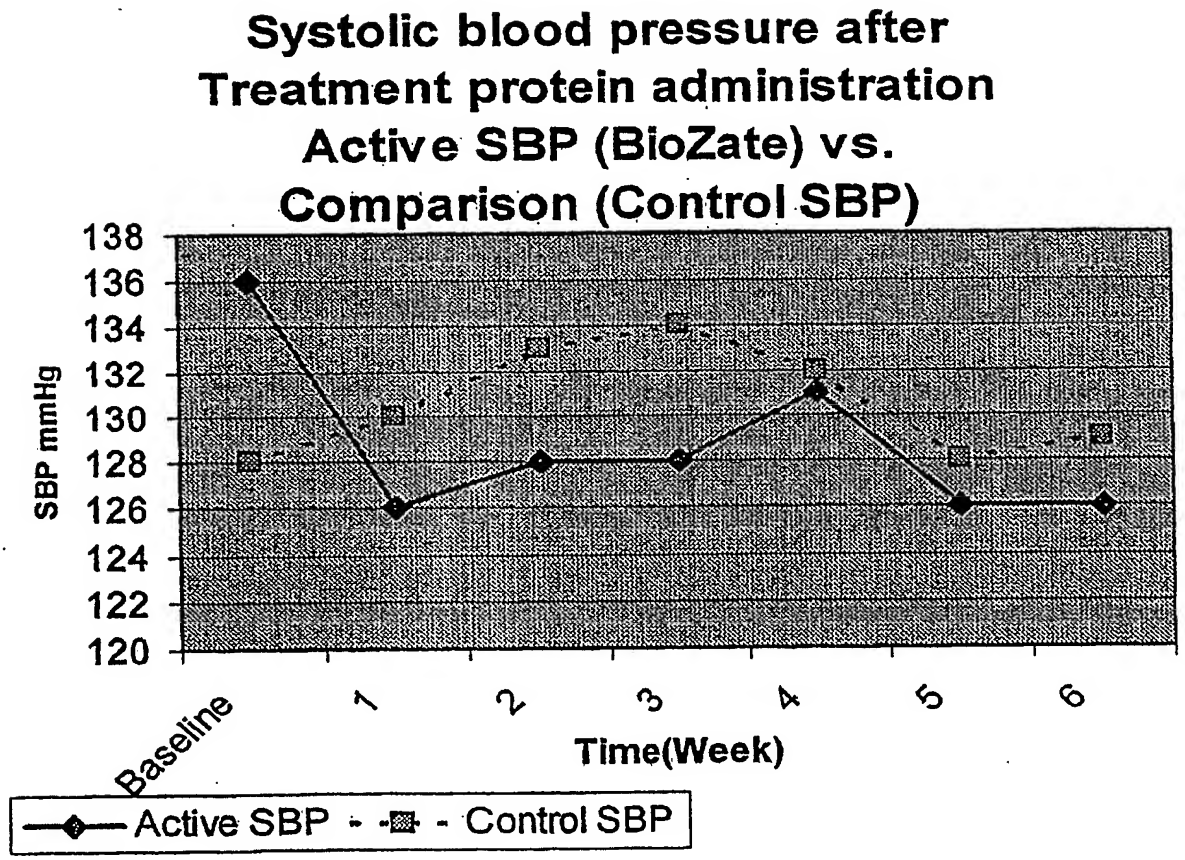
Systolic blood pressure after
Treatment protein administration
Active SBP (BioZate) vs.
Comparison (Control SBP)
SBP mmHg
138
136
134
132
130
128
126
124
122
120
Baseline
1
2
3
4
5
6
Time(Week)
Active SBP
Control SBP

FIG. 7

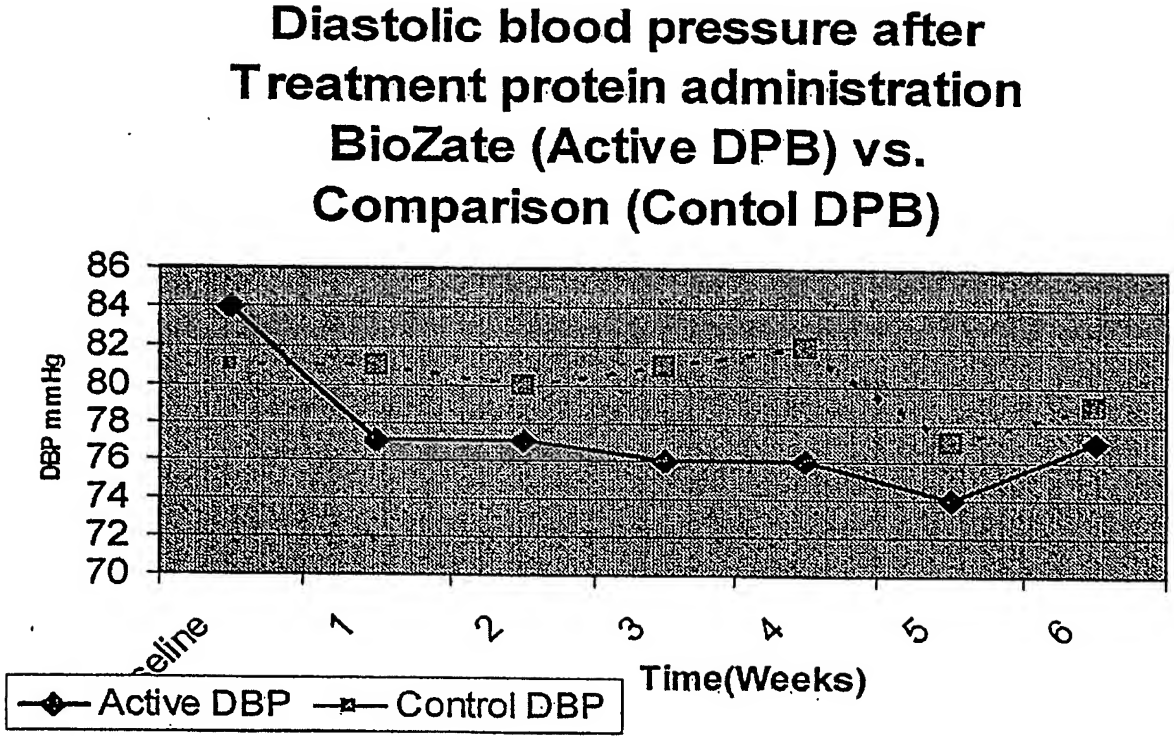
Diastolic blood pressure after Treatment protein administration BioZate (Active DPB) vs. Comparison (Contol DPB)
86
84
82
80
78
76
74
72
70
DBP mmHg
1
2
3
4
5
6
Time(Weeks)
Active DBP
Control DBP

FIG. 8

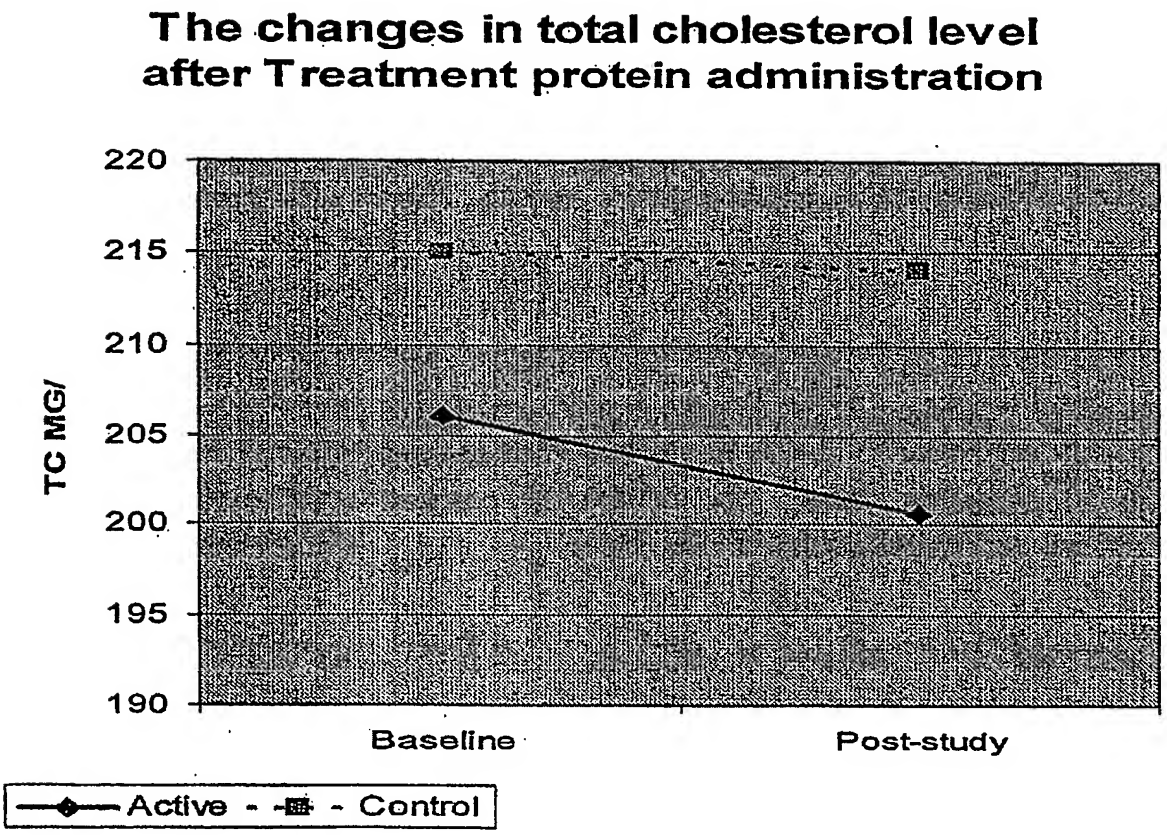
The changes in total cholesterol level after Treatment protein administration
220
215
210
205
200
195
190
TC MG/
Baseline
Post-study
Active
Control

FIG. 9

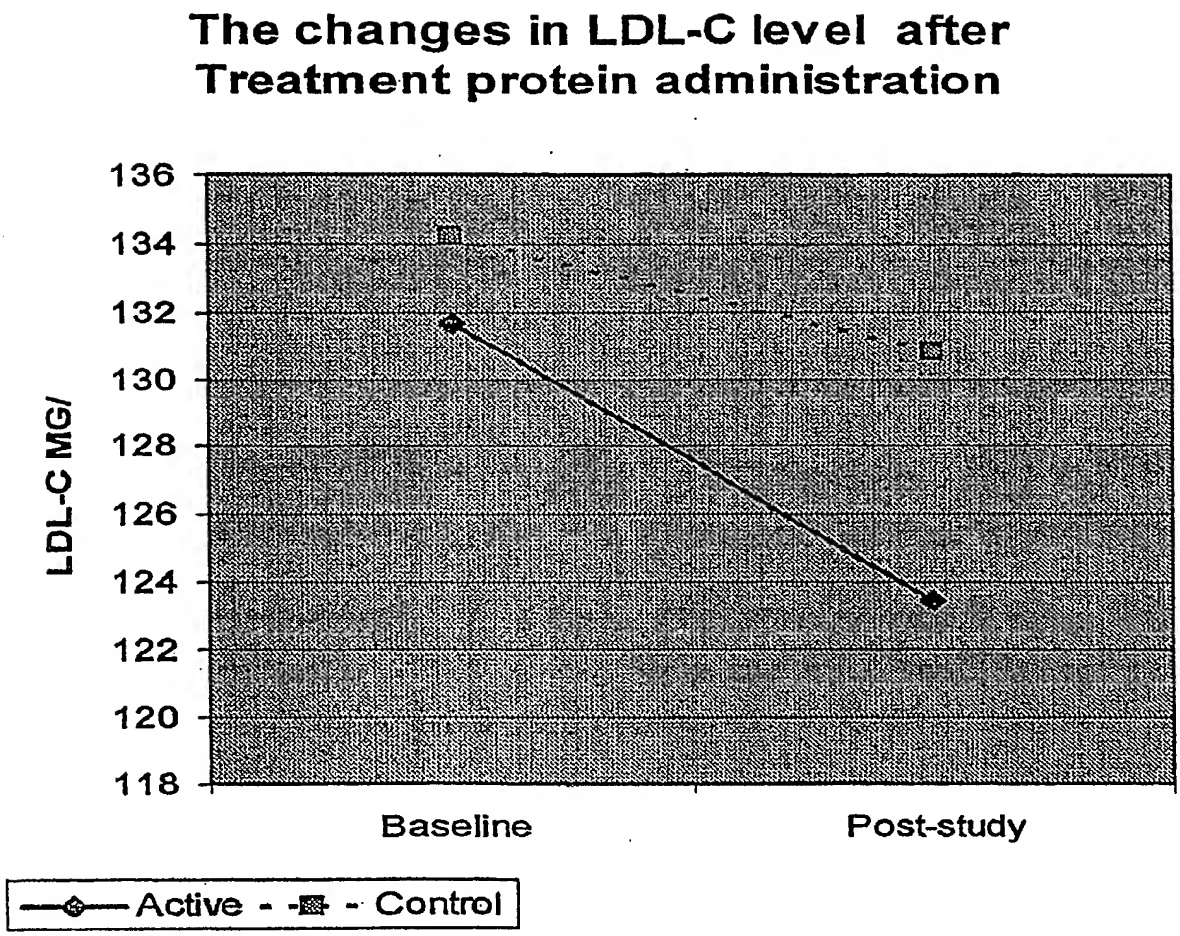
The changes in LDL-C level after Treatment protein administration
LDL-C MG/
136
134
132
130
128
126
124
122
120
118
Baseline
Post-study
Active
Control

FIG. 10

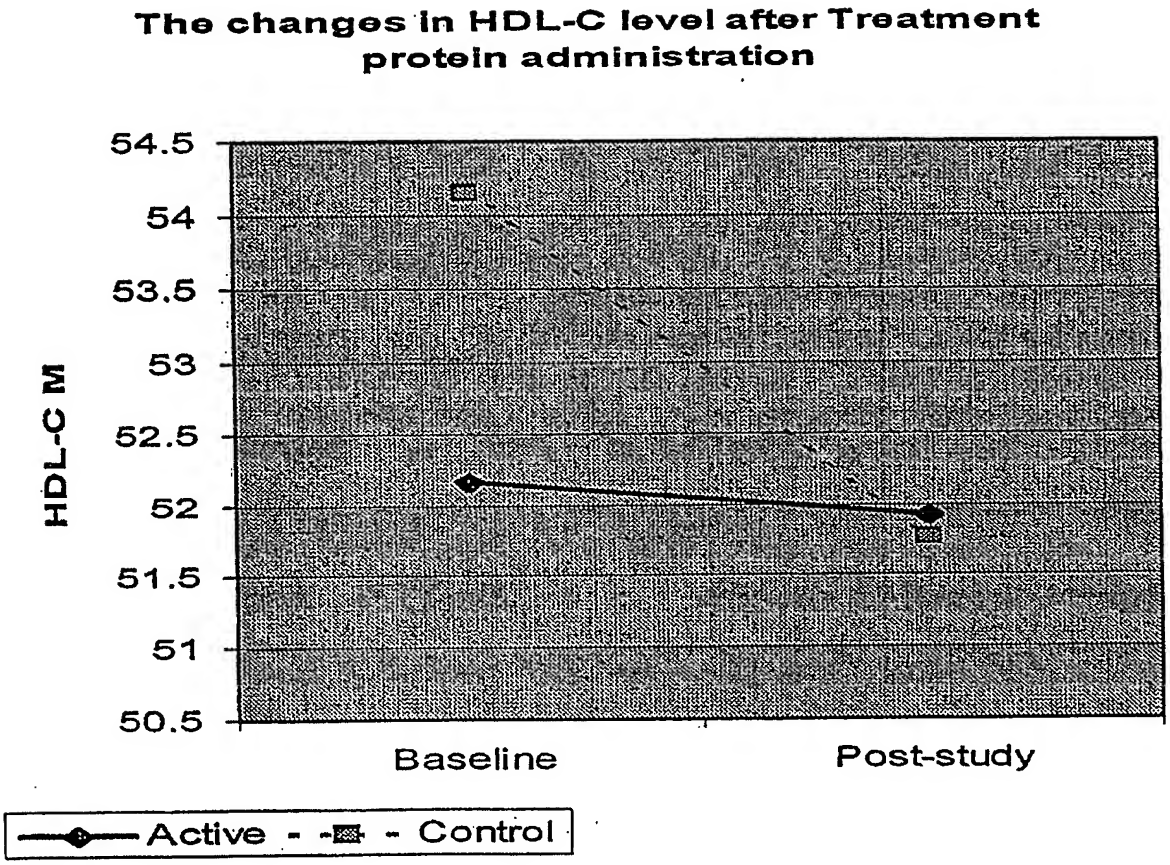
The changes in HDL-C level after Treatment protein administration
HDL-C M
54.5
54
53.5
53
52.5
52
51.5
51
50.5
Baseline
Post-study
Active
Control

FIG. 11

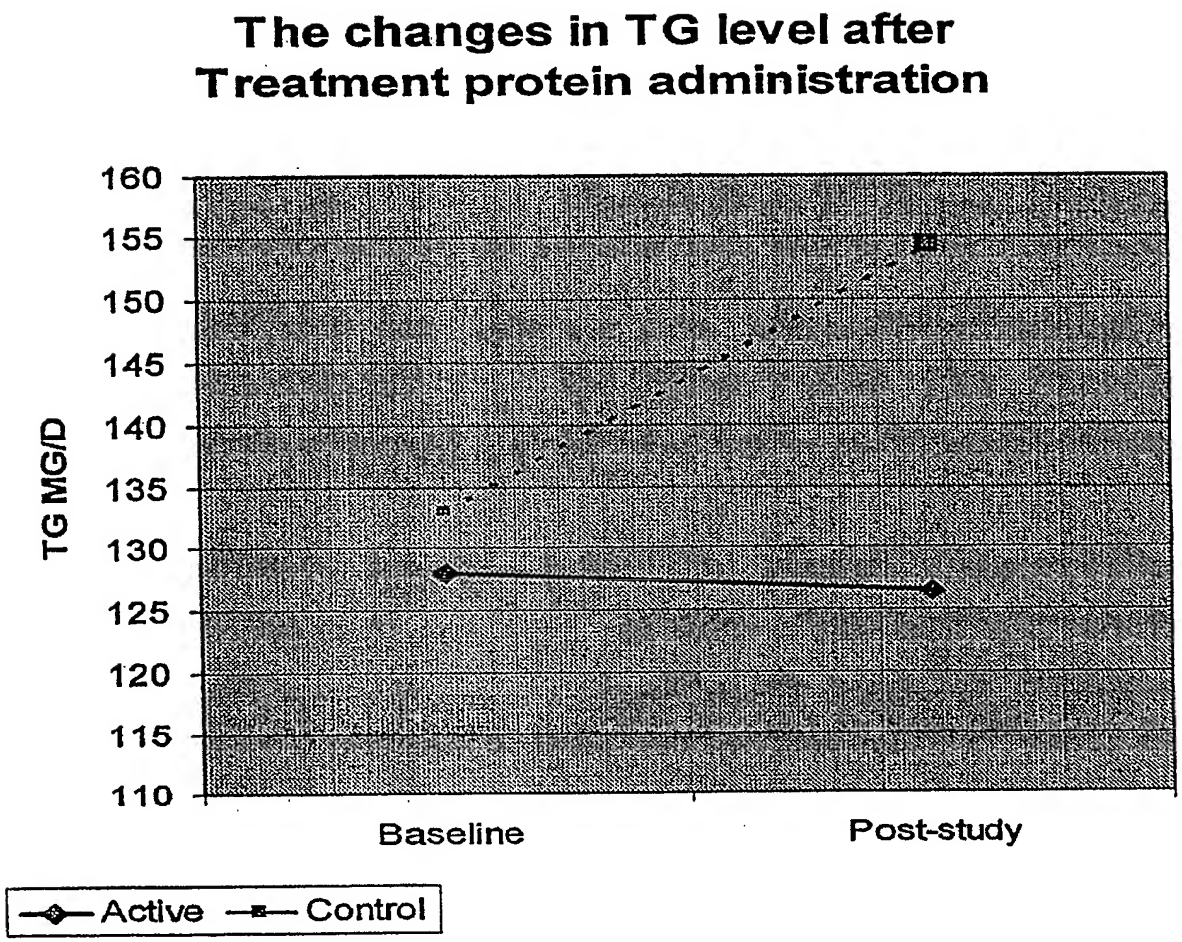
The changes in TG level after
Treatment protein administration
160
155
150
145
140
135
130
125
120
115
110
TG MG/D
Baseline
Post-study
Active
Control

FIG. 12

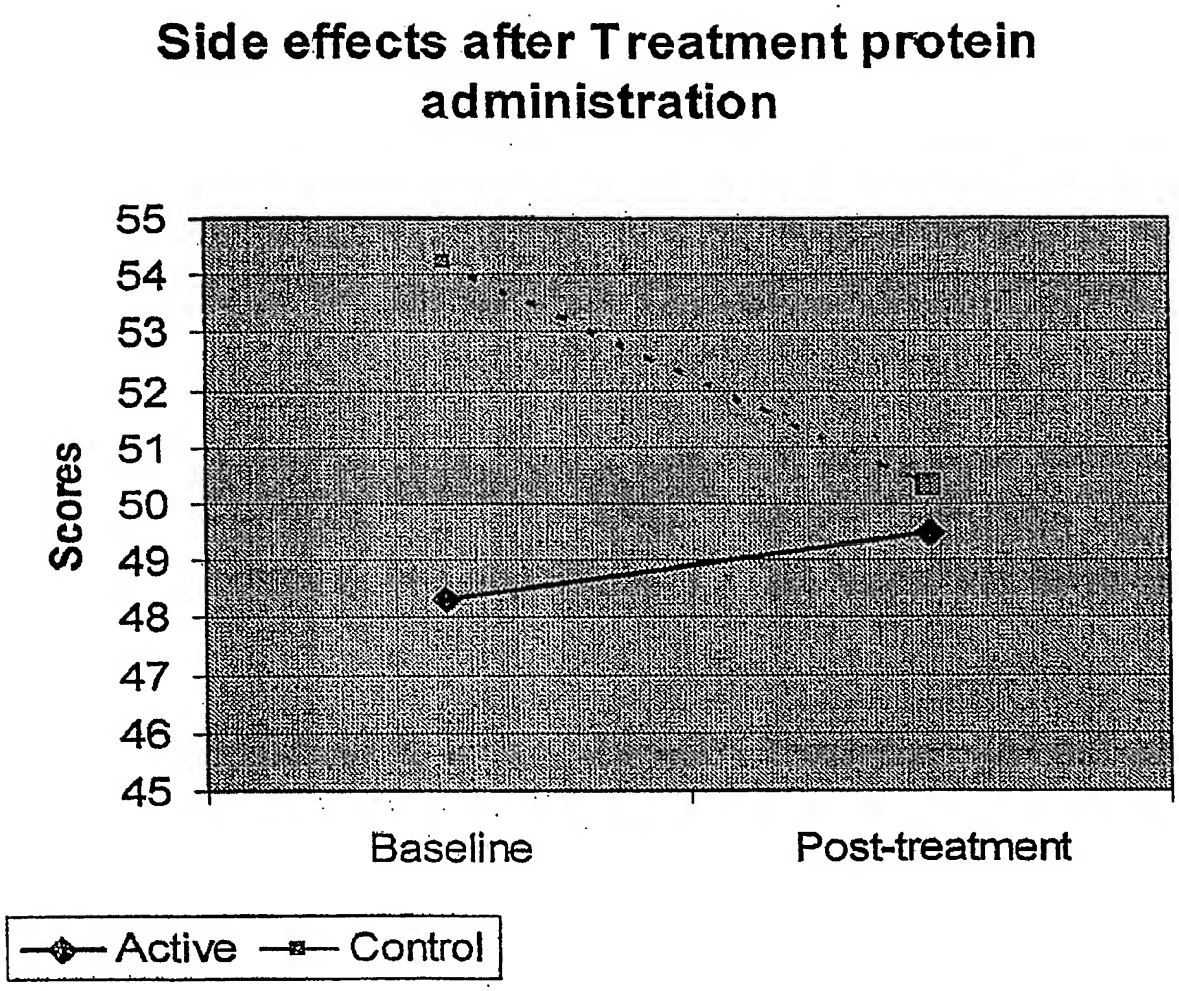
Side effects after Treatment protein administration
Scores
55
54
53
52
51
50
49
48
47
46
45
Baseline
Post-treatment
Active
Control

FIG. 13

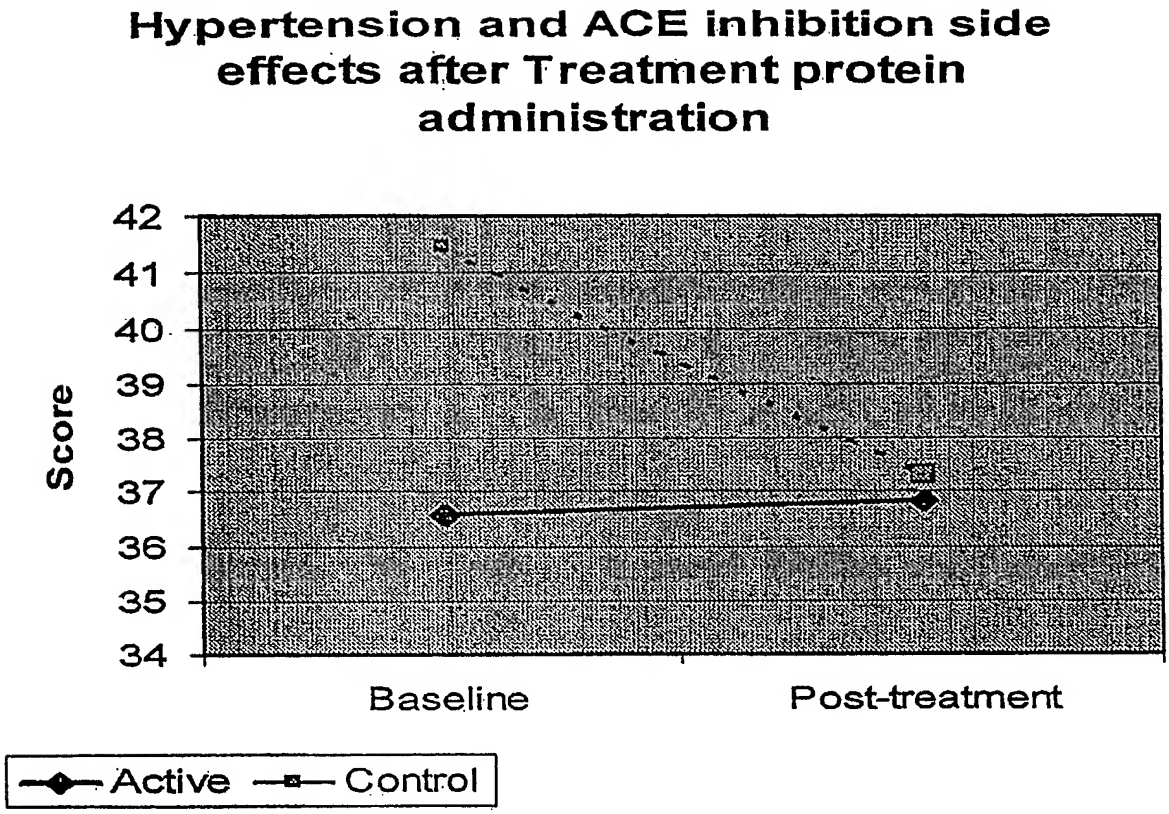
Hypertension and ACE inhibition side effects after Treatment protein administration
Score
42
41
40
39
38
37
36
35
34
Baseline
Post-treatment
Active
Control

FIG. 14

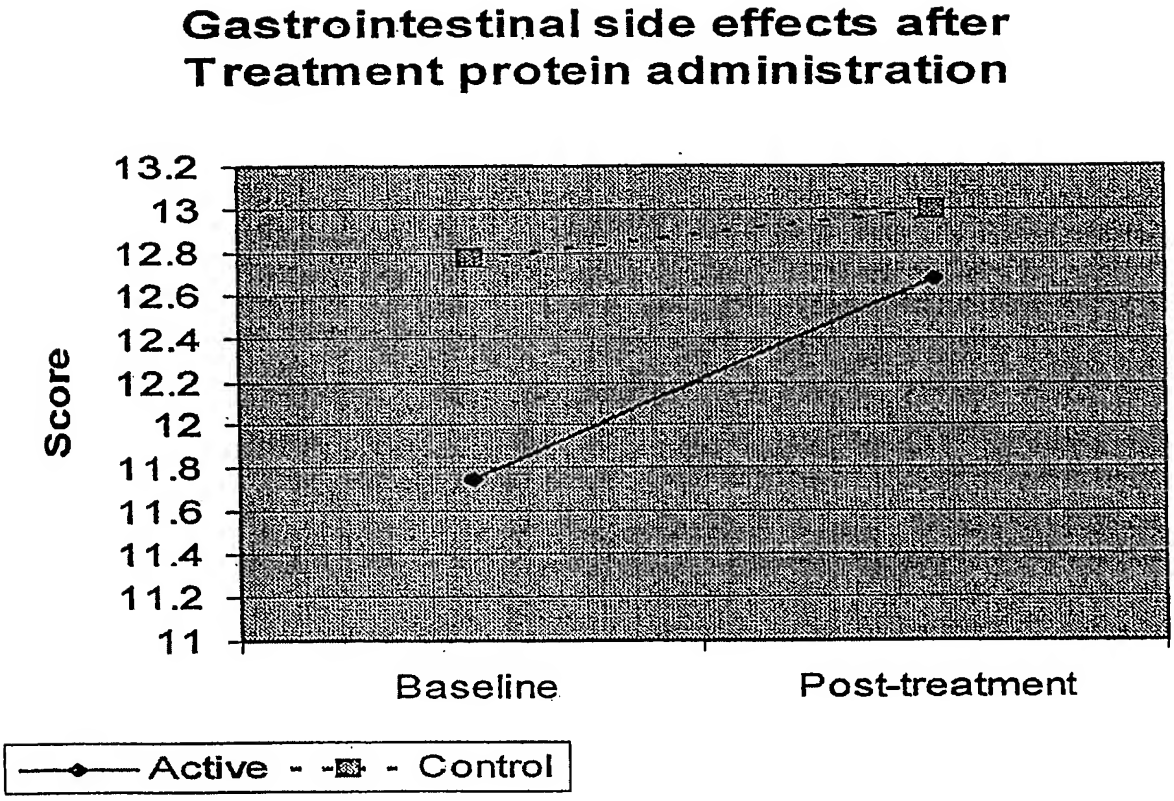
Gastrointestinal side effects after Treatment protein administration
13.2
13
12.8
12.6
12.4
12.2
12
11.8
11.6
11.4
11.2
11
Score
Baseline
Post-treatment
Active
Control

FIG. 15

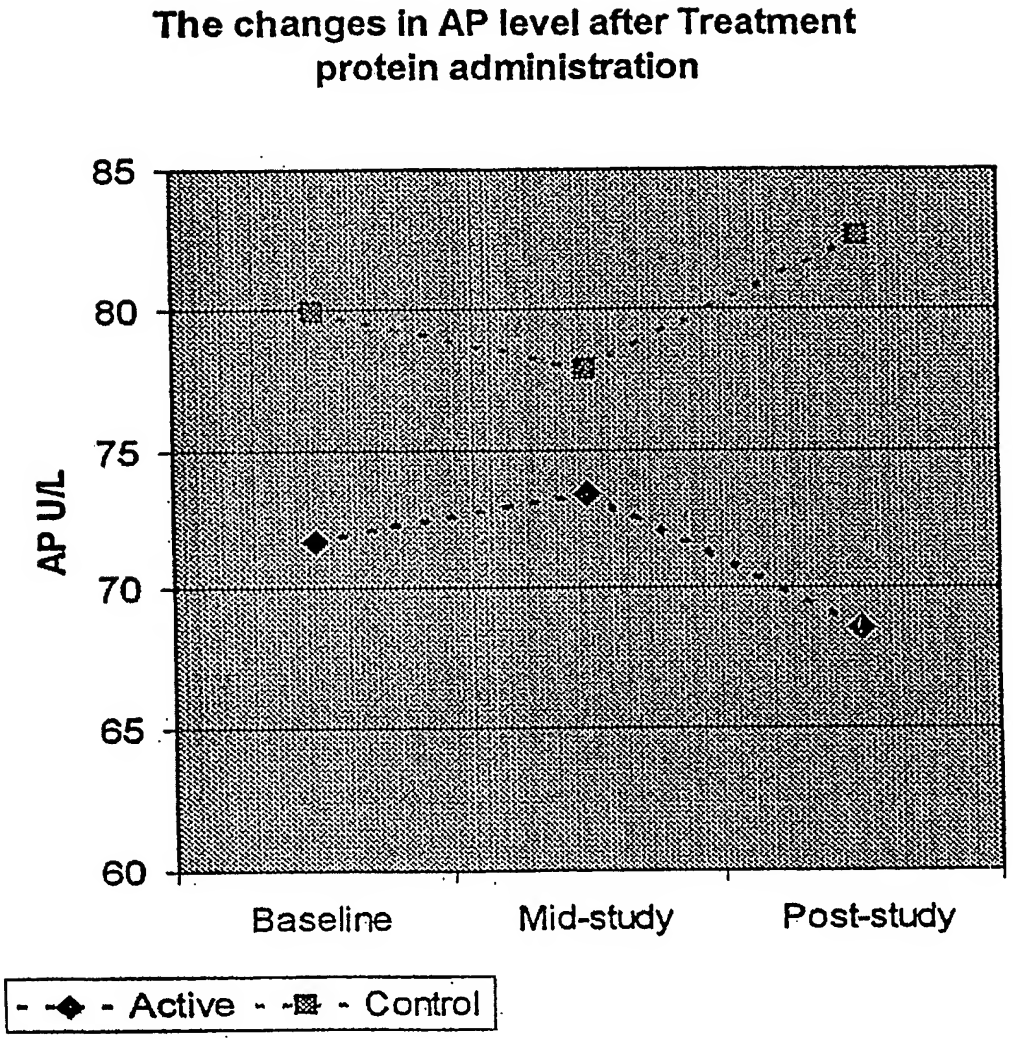
The changes in AP level after Treatment protein administration
85
80
75
70
65
60
AP U/L
Baseline
Mid-study
Post-study
Active
Control

FIG. 16

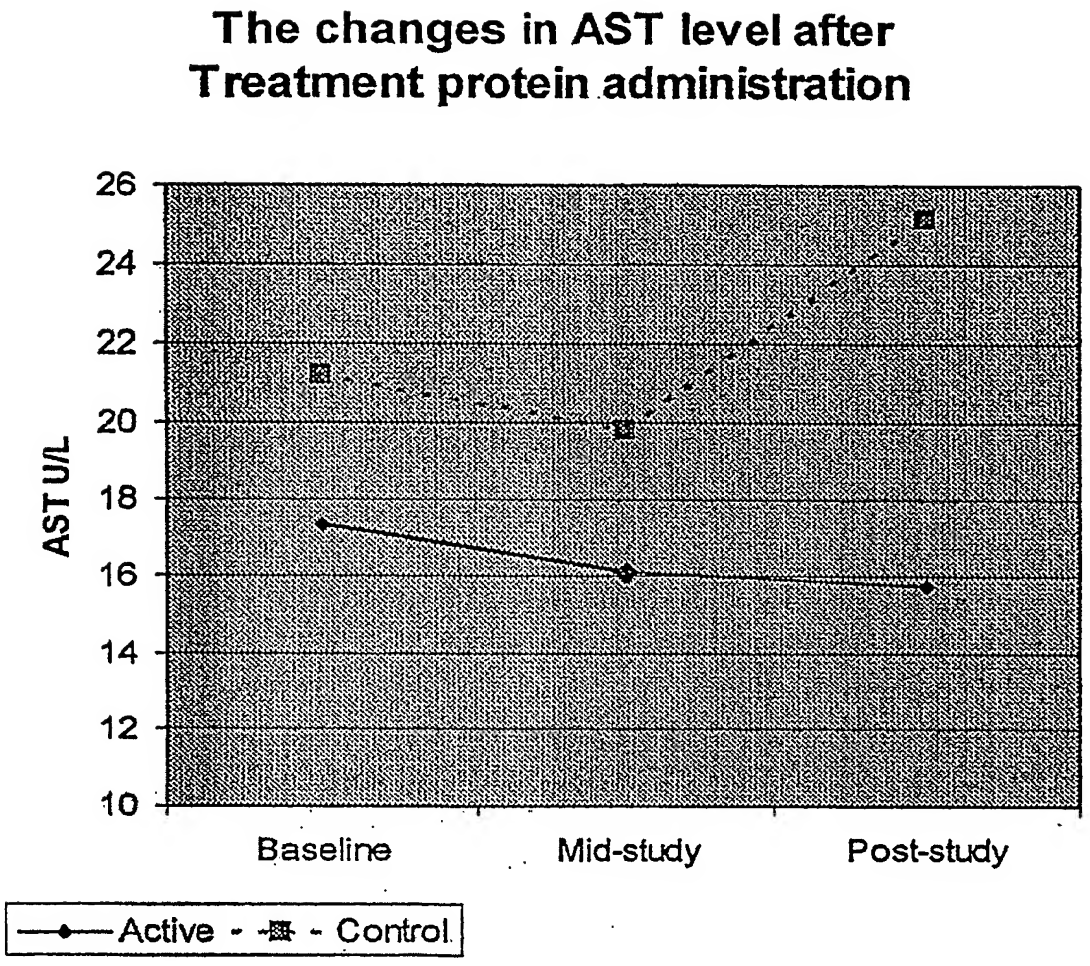
The changes in AST level after
Treatment protein administration
AST U/L
26
24
22
20
18
16
14
12
10
Baseline
Mid-study
Post-study
Active
Control

FIG. 17

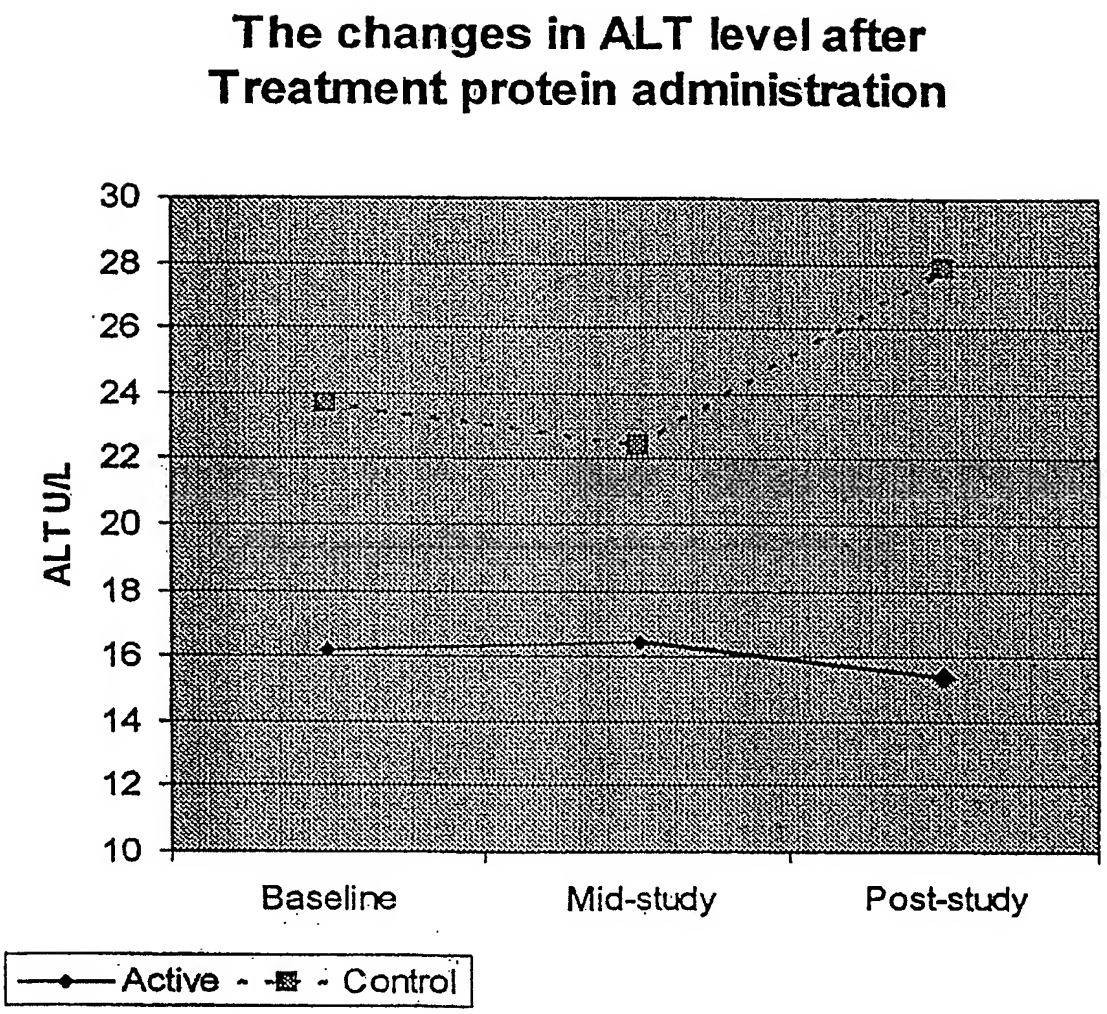
The changes in ALT level after Treatment protein administration
ALT U/L
30
28
26
24
22
20
18
16
14
12
10
Baseline
Mid-study
Post-study
Active
Control

FIG. 18

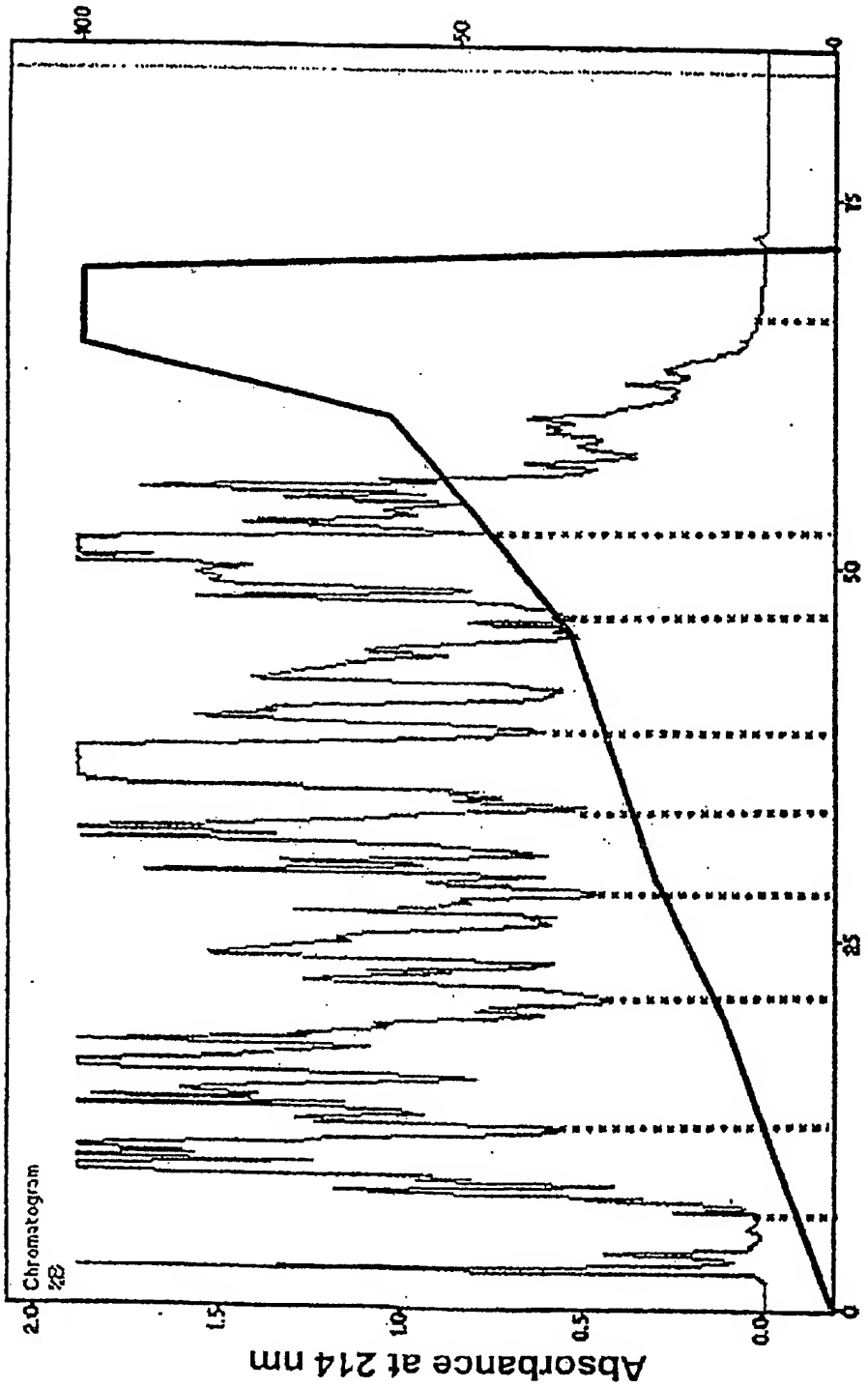
Absorbance at 214 nm
2.0
1.5
1.0
0.5
0.0
Chromatogram
%B
100
50
0
0
25
50
75
ELUTION TIME (MIN)

FIG. 19

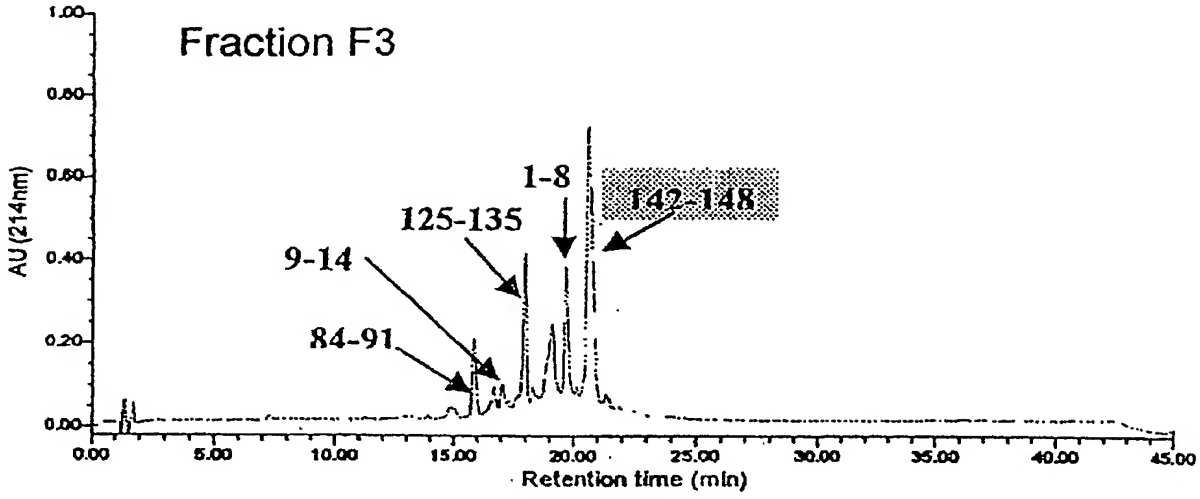

FIG. 20A

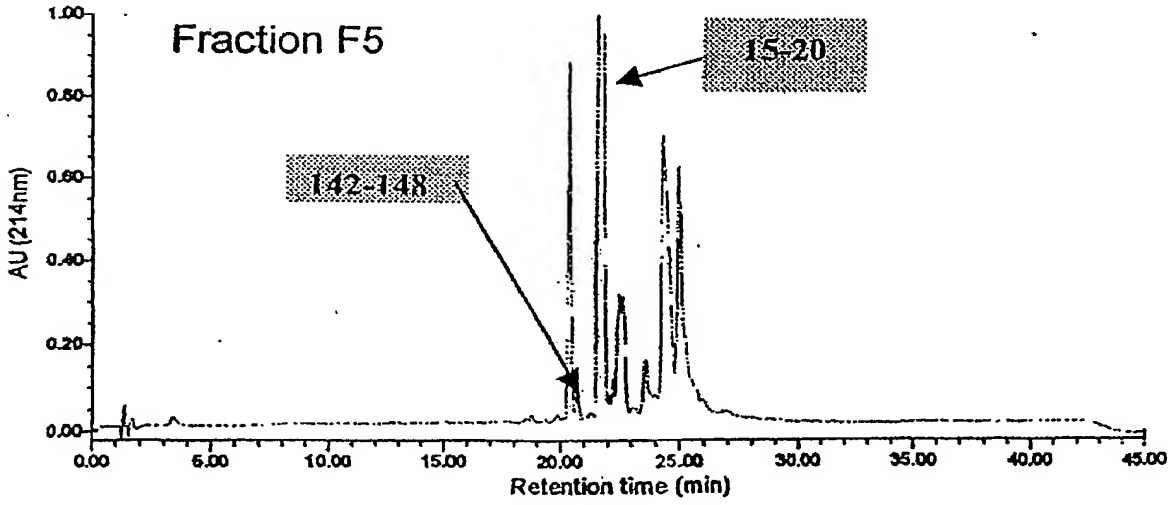

FIG. 20B